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POTENCY OF LIVER EXTRACT IN STIMULATING GASTRIC SECRETION BY INTRAVENOUS INJECTION AND BY DIRECT LAVAGE

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The possibility of a humoral or hormonal mechanism for the gastric secretory response to a meal was suggested by Edkins in 1906 (1). Subsequent work has furnished corroborative evidence for the existence of such a humoral mechanism (2, 3, 4, 5).

Kim and Ivy (6) obtained vaso-depressor free extracts of liver which contained potent secretagogues for gastric secretion. They also found that an isolated vagotomized pouch of the entire stomach could be made to secrete free acid by the slow intravenous injection of the extract, as well as by direct application to the gastric mucosa. According to their report, 400 mgm. of the extract was required to obtain a minimal response via the intravenous route while only 100 mgm. was required on direct gastric lavage. Thus a ratio of roughly 4:1 was established. These observations upon threshold dose were not supported by any detailed data or experimental protocols.

A series of sixty-three experiments were conducted to confirm these observations.

METHODS. Two dogs were prepared with vagotomized pouches of the entire stomach and esophago-jejunal anastomoses. The animals were fasted for ten to fourteen hours before each experiment. They were trained to stand in stocks while an experiment was in progress.

Basal secretion was collected for two and one-half hours and titrated against 0.0278 N NaOH(1 cc. equals 1 mgm. HCl) for free and total acid with Topfer's reagent and phenolphthalein. If any free acid was present during this period, the animal was not used for experimentation at that time.

The intravenous injections extended over exactly thirty minutes. They were given by slow continuous drip into the basilic vein. The gastric secretion, during the thirty minute injection period and for two hours following, was collected and titrated in the same manner as the basal secretion.

Direct gastric lavage was performed according to the method of Shoch and Bucher (7). This method consists of the insertion of a small rubber catheter

into the stomach pouch. The catheter is connected to one outlet of a three-way stopcock. A second outlet of the stopcock is connected to a piece of flexible rubber tubing draining into a small beaker which is suspended directly under the stoma of the pouch. The third outlet of the stopcock is attached to a 50 cc. syringe. This method permits the solution to be perfused into the pouch and the overflow allowed to drain back into the beaker by the proper adjustment of the stopcock. The overflow is then aspirated into the syringe (by turning the stopcock) and reperfused into the pouch (by again turning the stopcock). This procedure is continued for the thirty minutes of the lavage period.

At the end of the thirty minute period, the perfusate is allowed to drain into the beaker as completely as possible, and is emptied into a graduated cylinder.

A large rubber catheter, with numerous side openings to allow for drainage, is then inserted into the pouch in place of the smaller catheter. Fifty cubic centimeters of distilled water is then perfused into the pouch and allowed to drain out immediately into the beaker. This washing is combined with the perfusate and the volume measured.

Post-lavage secretion is collected for two hours and titrated in the same manner as for intravenous administration.

It is not possible to use accurately direct colorimetric titration for the lavage solution due to: 1, the color of the solutions; 2, the fact that the extract solution before lavage is not at neutrality; 3, the possible buffering action of the various constituents of the solution.

The pH of a sample of the original extract solution is determined by use of the glass electrode potentiometer. The pH of the lavage solution is determined and the solution titrated back to its original pH value with 0.0278 N NaOH (1 cc. equals 1 mgm. HCl).

This method of estimating acid output has been found reliable by studies with solutions of known acid content.

The liver extract used in this series of experiments was from a single batch of Lederle's Liver Concentrate, 15 Anti-Pernicious-Anemia units per cc.[1] The extract contained no phenol or preservatives, and was filtered through a large Berkefeld filter and stored on ice in large sealed serum bottles. This extract was found to contain no vasodilatins by blood pressure studies on anesthetized dogs and action upon isolated guinea-pig intestinal strips.

Measured amounts of the extract were diluted to 50 cc. with normal saline immediately before use. The saline was prepared from freshly distilled water before each experiment. The solution was then filtered through two thicknesses of No. 500 filter paper.

Graduated amounts of the extract were used so that the minimal stimulating dose could be determined. The secretory response, in terms of total acid output, caused by different dosages of the liver extract is presented in the accompanying table.

Results. It is evident from the data shown in table 1 that secretagogues applied directly to the stomach are more potent than secretagogues injected

[1] The liver extracts were generously provided by Dr. Y. Subbarow of Lederle, Inc.

intravenously. In dog 4, the lavage threshold dose is approximately 2.0 cc. of extract, while the intravenous threshold dose lies between 10 cc. and 20 cc. of extract. In dog 6, the lavage threshold dose is about 2.0 cc. of extract while the intravenous threshold dose is 5.0 cc.

TABLE 1

Total acid

GASTRIC LAVAGE			INTRAVENOUS		
Dose extract cc.	Total acid Mgm. HCl	Trials	Dose extract cc.	Total acid mgm. HCl	Trials
		Dog 4			
2.0	0.1	2	5.0	1.8	3
3.0			10.0	1.2	3
4.0	6.8	1	20.0	0.9	2
5.0	5.8	3	30.0	5.2	2
6.0	27.5	5	40.0	43.9	2
7.0	14.4	3			
8.0	5.1	3			
9.0					
10.0					
11.0	22.6	1			
12.0					
13.0					
14.0	17.0	1			
Total trials		19			12
		Dog 6			
2.0	1.0	2	5.0	0.0	1
3.0	13.5	2	10.0	3.8	3
4.0	17.0	3	20.0	10.5	2
5.0	9.7	2	30.0	21.8	2
6.0	27.3	3	40.0	14.7	2
7.0	34.7	3			
8.0	36.4	3			
9.0	39.9	1			
10.0					
11.0	41.9	2			
12.0					
13.0					
14.0	46.6	1			
Total trials		22			10

The reported observation of a ratio of 4:1 for threshold doses (6) is not confirmed by these data; the ratio is subject to variation between dogs. However, the more important observation of Kim and Ivy (6) is confirmed, namely, that secretagogues are more potent when applied to the gastric mucosa than when injected intravenously.

DISCUSSION. A humoral mechanism of gastric secretion has been established (2, 3, 4, 5). This humoral mechanism for the gastric phase of gastric secretion involves one of three general processes: 1, gastric secretatogues, present in foods, pass directly through the gastric mucosa and pass via the blood and lymph channels to the gastric secretory mechanism; 2, gastric secretagogues, present in foods, become altered in the stomach wall and then pass into the blood and lymph in an altered form to reach the gastric secretory mechanism; 3, gastric secretagogues, present in foods, stimulate the formation of a new substance, a hormone, in the gastric mucosa or submucosa which passes into the blood and lymph and stimulates the gastric secretory mechanism.

CONCLUSION

The results of this study indicate that secretagogues do not act by passing directly into the blood in an unchanged form, but act either in an altered form, the alteration being due to gastric juice, or by stimulating the elaboration of a gastric hormone.

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STUDIES ON THE EFFECT OF THYMOXYETHYLDIETHYLAMINE (929 F) AND N-DIETHYLAMINOETHYL-N-ETHYLANILINE ON GASTRIC SECRETION IN THE DOG[1]

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Bovet and Staub (1937) reported that thymoxyethyldiethylamine (929 F) antagonized some of the physiologic actions of histamine. Staub (1939) found that N-diethylaminoethyl-N-ethylaniline (1571 F) was, in some of its actions, a more active antagonist of histamine than 929 F. Loew and Chickering (1941) found that 929 F did not decrease but significantly *increased* the gastric secretory response to histamine from Heidenhain pouches in dogs. Bourque and Loew (1943) reported that 1571 F reduced gastric secretion after stimulation by both histamine and pilocarpine, but failed to influence the gastric secretory response to a meal from dogs that had Heidenhain pouches. Burchell and Varco (1942) found that neither 929 F nor 1571 F affected the secretory response of dogs that had Heidenhain pouches to histamine.

The present investigation was carried out to determine the effect of 929 F and 1571 F on the normal gastric secretory process by studying their action on the secretory response to a meal. The effects of 929 F on the gastric secretory response to mecholyl chloride and to small doses of histamine have also been studied.

METHODS. Gastric secretion was studied in ten dogs. Four had pouches of the Pavlov type (Gregory, Hallenbeck and Code, 1942), four had Heidenhain pouches and two had transplanted gastric pouches. The animals were fasted for eighteen to twenty-four hours before collections of gastric juice were begun.

Mecholyl chloride was administered at constant rates by the continuous intravenous drip method. Histamine diphosphate was administered both in dilute solution by the continuous intravenous drip method and by intramuscular injection of a mixture of histamine, beeswax and sesame oil, which resulted in slow absorption and prolonged action of the histamine (Code and Varco, 1942). Doses of histamine are expressed in terms of the base. 929 F and 1571 F were provided by the Abbot Laboratories. Dr. M. H. Power and Dr. H. L. Mason kindly analyzed these compounds for their chlorine and nitrogen content and determined their melting points. The 929 F was found to be pure. The 1571 F was found to be pure except for the presence of 8.1 per cent of water by weight. Doses of 929 F are expressed as the monohydrochloride; doses of 1571 F are expressed as the dihydrochloride.

Doses of 929 F varied from 1 to 4 mgm. per kilogram of body weight per hour

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D. in Physiology. The work on which this paper is based was done at the Institute of Experimental Medicine of the Mayo Foundation.

when the drug was given intravenously by continuous drip, and from 2 to 4 mgm. per kilogram per hour when it was given subcutaneously. These doses of the drug were subemetic. Slightly larger doses produced emesis. 1571 F was given by continuous intravenous drip in doses of 15 to 20 mgm. per kilogram per hour. With these and larger doses the drug was not emetic when given intravenously. The larger doses, however, produced a state of increased irritability suggestive of stimulation of the central nervous system. Neither drug caused elevation of body temperature.

In experiments in which the gastric secretory response to a meal was studied, the secretory stimulant consisted of raw lean horse meat. The weight of meat fed was constant for each dog throughout the study, ranging from 100 to 300 grams for the different dogs.

Samples of gastric juice were collected at thirty-minute intervals. When it had been established that fasting secretion was not present in amounts exceeding a fraction of a cubic centimeter, the secretory stimulus was given. The volume of each sample was determined and aliquots were titrated for free acid, Töpfer's reagent being used as the indicator.

RESULTS. 1. *The effect of 929 F on the gastric secretory response to histamine.* In tests on two dogs that had Heidenhain pouches, gastric secretion stimulated by histamine given by continuous intravenous drip at a dose of 150 micrograms per hour was not inhibited by the subcutaneous injection of 929 F. Indeed, in the majority of the tests the output of gastric juice was greater after administration of the 929 F than before its administration. A similar result was obtained when the histamine was given intramuscularly in a mixture with beeswax and sesame oil. These results are in agreement with the earlier findings of Loew and Chickering (1941).

2. *The effect of 929 F on the response of Heidenhain pouches to mecholyl chloride.* As Gray and Ivy (1937) have shown, there exists for a given dog a dose of mecholyl chloride which is optimal for stimulation of gastric secretion. Such optimal doses of the drug, given intravenously by continuous drip, were established for each of four dogs that had Heidenhain pouches. The volume and free acidity of the gastric juice secreted during the first two hours of the response to mecholyl chloride were compared with those of the juice secreted during the first two hours of the response to a mixture of mecholyl chloride and 929 F. In the latter case the 929 F was first given alone in physiologic saline solution for thirty minutes, and then the mecholyl chloride was added to the solution and the two were given simultaneously.

Twenty-six tests were conducted on the four dogs that had Heidenhain pouches. Three of the four dogs showed diminished output of juice and hydrochloric acid, and one of the dogs showed an increased output of juice when 929 F was given (table 1). The differences between the weighted mean values for the four dogs were 1.7 per cent for volume of juice and 8.5 per cent for output of hydrochloric acid, both figures indicating a decrease of secretion after administration of 929 F. However, the standard errors of the various mean differences were large. In no case was a mean difference as much as three times the magni-

tude of its standard error, the criterion here accepted to indicate significance of the figure. Thus, although three of the four dogs showed diminished volume and output of acid with 929 F, the magnitude of this reduction was definitely within the limits of the control observations. It has been concluded, therefore, that 929 F does not have any significant effect on the gastric secretory response to mecholyl chloride from Heidenhain pouches in dogs.

3. *The effect of 929 F and 1571 F on the gastric secretory response to a meat meal.* Secretory responses to a meal were studied in four dogs that had Pavlov pouches, two dogs that had Heidenhain pouches and two dogs that had transplanted gastric pouches. During some of the control trials in all of the animals physiologic saline solution was given intravenously by the continuous drip method at a rate of 50 cc. per hour for the first one or one and a half hours after the meal was fed. This procedure duplicated the conditions of the trials in which either 929

TABLE 1

The effect of 929 F on the first two hours of the gastric secretory response to mecholyl chloride of four dogs that had Heidenhain pouches

DOG	EXPERIMENTS		DIFFERENCE BETWEEN SECRETION WITHOUT 929 F AND THAT WITH 929 F			
	Without 929 F	With 929 F	In volume of secretion		In output of hydrochloric acid	
			Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
1	3	3	−0.3 ± 2.5*	−3.3	−7.3 ± 8.0	−33.3
2	3	3	−4.6 ± 3.6	−34.6	−25.4 ± 20.5	−48.7
3	4	4	+4.0 ± 11.2	+12.0	+24.5 ± 67.5	+15.4
4	3	3	−1.9 ± 3.5	−11.3	−27.4 ± 16.8	−41.1
Total...	13	13	−0.3 ± 3.2	−1.7	−6.3 ± 18.2	−8.5

* Standard error.

F or 1571 F was used. In no case did administration of physiologic saline solution alone inhibit the gastric secretory response to the meat meal.

(a) *Pavlov pouches.* 929 F and 1571 F inhibited the gastric secretory response to a meal in dogs that had Pavlov pouches. In general, such inhibition became evident about thirty minutes after the administration of either of the drugs had been started. Maximal inhibition usually persisted for a half to one hour after the administration of either of the drugs had been stopped. During the following two hours, the rate of secretion gradually increased. During the first two hours after ingestion of the meal the inhibition of volume of juice and output of hydrochloric acid after administration of 929 F varied from about 30 per cent to about 80 per cent of the control values, while that for 1571 F varied from about 65 per cent to 90 per cent of the control values (tables 2 and 3). The differences were found to be statistically significant.

(b) *Heidenhain pouches.* One dog (Heidenhain 1, table 4) received 929 F subcutaneously in a dose of 2 mgm. per kilogram of body weight at the time of feeding. The volume of juice and output of hydrochloric acid were reduced

TABLE 2

The effect of 929 F on the first two hours of the gastric secretory response of pouches of the Pavlov type to a meat meal

DOG	EXPERIMENTS		DIFFERENCE BETWEEN SECRETION WITHOUT 929 F AND THAT WITH 929 F			
	Without 929 F	With 929 F	Reduction of volume of secretion		Reduction of output of hydrochloric acid	
			Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
1	7	4	35.0 ± 5.0*	71.6	194.6 ± 25.3	78.5
2	12	3	27.6 ± 4.4	46.2	157.8 ± 28.6	47.9
3	6	4	18.5 ± 4.7	68.3	101.7 ± 26.0	74.1
4	7	4	19.4 ± 4.5	31.6	106.8 ± 25.6	32.6
Total....	32	15	28.0 ± 1.8	54.3	158.2 ± 13.2	57.5

* Standard error.

TABLE 3

The effect of 1571 F on the first two hours of the gastric secretory response of pouches of the Pavlov type to a meat meal

DOG	EXPERIMENTS		DIFFERENCE BETWEEN SECRETION WITHOUT 1571 F AND THAT WITH 1571 F			
	Without 1571 F	With 1571 F	Reduction of volume of secretion		Reduction of output of hydrochloric acid	
			Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
1	7	2	36.3 ± 3.8*	74.2	197.2 ± 20.1	79.5
2	12	2	39.0 ± 2.6	65.3	234.9 ± 17.8	71.4
3	6	2	21.9 ± 4.6	80.8	122.9 ± 25.9	90.0
4	7	2	43.3 ± 4.3	70.5	245.8 ± 32.1	74.9
Total....	32	8	37.4 ± 3.7	72.5	214.7 ± 20.5	78.0

* Standard error.

TABLE 4

The effect of 929 F on the first two hours of the gastric secretory response of Heidenhain and transplanted gastric pouches to a meat meal

DOG	EXPERIMENTS		REDUCTION OF VOLUME OF SECRETION		REDUCTION OF OUTPUT OF HYDROCHLORIC ACID	
	Without 929 F	With 929 F	Mean difference	Per cent	Mean difference	Per cent
			cc.		*mgm.*	
Heidenhain 1.....	4	3	6.2 ± 1.8*	36.7	31.5 ± 14.8	45.9
Heidenhain 2.....	4	3	8.9 ± 1.7	65.4	49.9 ± 10.1	83.0
Transplant 1.....	3	1	4.6	59.0	11.9	100.0
Transplant 2.....	4	4	11.6 ± 0.5	80.6	60.5 ± 1.5	89.1

* Standard error.

36.7 and 45.9 per cent respectively. The other dog (Heidenhain 2, table 4) received 929 F by continuous intravenous drip for one hour after feeding. The mean inhibition of the volume of juice secreted during the first two hours was 65.4 per cent; that of the output of hydrochloric acid was 83.0 per cent. With one exception, all mean differences were statistically significant.

(c) *Transplanted gastric pouches.* One dog (transplant 1, table 4) was given multiple subcutaneous injections of 929 F at intervals after ingestion of the meal. No free hydrochloric acid was secreted during the first two hours, a phenomenon which did not occur in three trials without the drug. The other dog (transplant 2, table 4) received 929 F by continuous intravenous drip for one hour after feeding. The volume of juice and the output of hydrochloric acid were inhibited 80.6 and 89.1 per cent respectively when 929 F was given. The mean differences were statistically significant.

Thus, throughout these experiments 929 F uniformly inhibited the secretory response of Pavlov, Heidenhain and transplanted gastric pouches to the ingestion of a meat meal. Likewise, 1571 F inhibited the secretory response of Pavlov pouches to the ingestion of a meat meal. The action of 1571 F on the response of Heidenhain and transplanted gastric pouches to a meat meal was not tested.

One dog that had a transplanted gastric pouch was prepared with a Mann-Bollman fistula of the stomach so that simultaneous records of gastric secretion and motility could be obtained. The secretory response of the pouch was inhibited as much as 80 per cent by 929 F without inhibition of gastric motility. This observation plus the finding in another study that inhibition of gastric motility by 929 F is inconstant (Hallenbeck, Code and Mann) indicates that inhibition of gastric motility plays a minor rôle, if any, in the inhibitory action of 929 F on the gastric secretory response to a meal.

COMMENT. The results after administration of 1571 F indicate that this drug inhibits the gastric secretory response of Pavlov pouches to the ingestion of a meat meal. Bourque and Loew (1943), however, observed that 1571 F failed to influence the response of Heidenhain pouches to a meal. The difference in the results is probably due to the difference in the nerve supply of the two types of pouches.

There is a possibility that part of the inhibition exercised by 929 F on the response of Pavlov pouch dogs to a meal may be the result of depression of psychic stimulation by the nausea which the drug may cause. Atkinson and Ivy (1938) have shown that apomorphine, emetine hydrochloride and quinine in emetic and subemetic doses can inhibit the gastric secretory response to histamine in dogs that have Pavlov pouches. However, the fact that the response to a meal is also inhibited in Heidenhain and transplanted pouch preparations in dogs treated with 929 F shows that inhibitory stimuli or diminution of psychic secretory stimuli reaching the gastric glands of the pouches via nerves is not an all-important factor.

The data in this report do not suggest that the inhibition of the gastric secretory response to a meal in dogs by 929 F and 1571 F is the result of direct antagonism of these drugs to histamine.

SUMMARY AND CONCLUSIONS

A study was made of the effect of 929 F and 1571 F on the secretory response of gastric pouches in dogs. The results were as follows:

1. The finding of other investigators that 929 F does not inhibit the secretory response of Heidenhain pouches to histamine was confirmed.

2. 929 F did not significantly alter the gastric secretory response of Heidenhain pouches to mecholyl chloride.

3. 929 F inhibited the gastric secretory response of Pavlov, Heidenhain and transplanted pouches to the ingestion of a meat meal.

4. 1571 F inhibited the gastric secretory response of Pavlov pouches to the ingestion of a meal.

I should like to express my gratitude to Dr. Charles F. Code for his help and guidance throughout the course of this study.

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THE EFFECTS OF HYPERVENTILATION AND OF BLOOD-PRESSURE CHANGES ON THE SELF-SUSTAINED RESPONSES OF THE CEREBRAL CORTEX

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The marked influence of hyperventilation on the spontaneous activity of the cerebral cortex has been repeatedly studied and over-breathing tests have become a routine procedure in clinical electro-encephalography. Nevertheless, very little is known about the action of CO_2 changes on the provoked cortical responses. Information on this point might bring some light on several obscure points.

Brody and Dusser de Barenne (1932) studied the effect of hyperventilation on the responses to electrical stimulation of the cortex by recording the muscular contraction provoked by the stimuli. With this procedure, however, several structures intervene between the cortex and the reacting muscles, that might react in different ways to the hyperventilation.

As opposed to this indirect method of approach, the study of the electrical self-sustained "tonic-clonic" responses recently analyzed by Rosenblueth and Cannon (1942) gives direct evidence about some cortical properties and their variations under different experimental conditions.

METHOD. Cats were used. In most of the experiments dial (Ciba, 0.65 cc. per kgm. intraperitoneally) was the anesthetic. Some animals were studied after a mesencephalic transection (Bremer, 1935a); in these cats a short period of ether anesthesia preceded the transection; bleeding during the operation was prevented by intermittent occlusion of the carotid arteries.

Tracheal cannulae with a side hole were used. Hyperventilation was induced by increasing the amount of air delivered or by speeding up the frequency of an artificial-respiration pump. A double action (suction and thrust) pump was used in order to give definite gas mixtures contained in Tissot gasometers.

The blood pressure was recorded in most of the experiments from the left carotid artery by means of a Hürthle manometer. Four per cent sodium citrate solution was employed for prevention of clotting and care was taken to prevent the animal receiving appreciable amounts of that solution during falls of blood pressure, since a large and sometimes irreversible decrease of cortical activity was observed when this occurred accidentally.

The cerebral hemispheres were exposed and the stimulating and recording electrodes (silver wires with a small bead at the tip) were applied to the surface of the pia. The interelectrodal distance was about 5 mm. both for stimulation and for recording. The stimuli were series of induced shocks from a Harvard coil with 3 volts on the primary. The maximal distance between the coils that

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yielded a well-developed "self-sustained response" was used. This distance was about 8 cm. in most cases. The duration of the trains was ordinarily 2 to 3 seconds and a constant interval of 2 to 3 minutes was allowed between successive periods of stimulation. Possible interfering facilitation and extinction factors (see Dusser de Barenne and McCulloch, 1939) were thus minimized.

The electrical phenomena in the cortex were recorded from 2 to 6 pairs of silver electrodes applied to the pia on different regions in one or both cerebral hemispheres. After capacity-coupled amplification the responses were recorded by means of ink-writing moving-coil galvanometers (six-channel Grass oscillograph). The amplifiers were operated in push-pull. The time constant was 0.15 second. The animal was grounded through a lead attached to the head-holder.

In the experiments in which occlusion of the abdominal aorta was performed a thread was passed under the artery above the renal arteries and both ends of the thread were then inserted into a piece of glass tubing; a hemostat supporting the thread on the free end of the tubing permitted an adequate regulation of the degree of occlusion. This device allowed closure of the abdominal cavity during the experiments.

RESULTS. A. *The self-sustained responses during hyperventilation.* We call "self-sustained responses" the periods of electrical events recorded from the cortex after adequate cortical stimulation. By extension of the term applied to the muscular contractions that under certain conditions accompany such cortical responses, these will also be called "tonic-clonic" (Rosenblueth and Cannon, 1942).

Hyperventilation with ordinary air invariably produced a decrease and eventually a suppression of the self-sustained responses. The time required for that effect was 1 to 5 minutes in most animals. The decrease in the self-sustained responses occurred not only as a shortening of the period of activity but also as a fall in voltage and a slowing of rate of the cortical discharges. In every instance either or both of these changes were evident. Thus the total energy consistently decreased (fig. 1). Increases were not observed.

The changes in cortical activity were reversible, provided the periods of hyperventilation were not too long. The recovery toward the original normal response was progressively slower after longer hyperventilation. Multiple leads from both cerebral hemispheres were used in order to study the influence of hyperventilation on the spread of the tonic-clonic responses. The changes were more or less parallel in all the leads (fig. 2).

A change of the type of the self-sustained responses to the cortical stimuli was sometimes observed during hyperventilation. The initial fast components decreased first and the clonic stage seemed to be relatively prolonged, although the total duration of the response was reduced.

The changes described were present when hyperventilation was tried after bilateral section of the vagi. Cats with a mesencephalic transection showed also similar changes and the same phenomenon was observed in animals in which blood-pressure changes were prevented, as will be described under C.

B. *The self-sustained responses with impaired cerebral circulation.* Blood-pressure records showed a marked fall during hyperventilation; an immediate

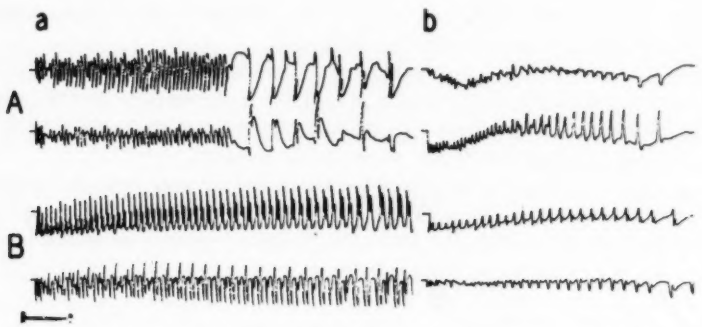

Fig. 1. Decrease of the self-sustained response of the cerebral cortex of the cat, after hyperventilation with ordinary air. The records begin shortly after the stimulus had been applied. This and the following electrical records were taken with capacity-coupled amplifiers and ink-writing galvanometers. Time calibration: 0.5 sec.

A. Responses to faradic stimulation (2 sec.), before, *a*, and after, *b*, 2 min. of hyperventilation.

B. The fall of blood pressure was prevented by compressing the abdominal aorta. Responses to the same faradic stimulations before, *a*, and after, *b*, 4 min. of hyperventilation.

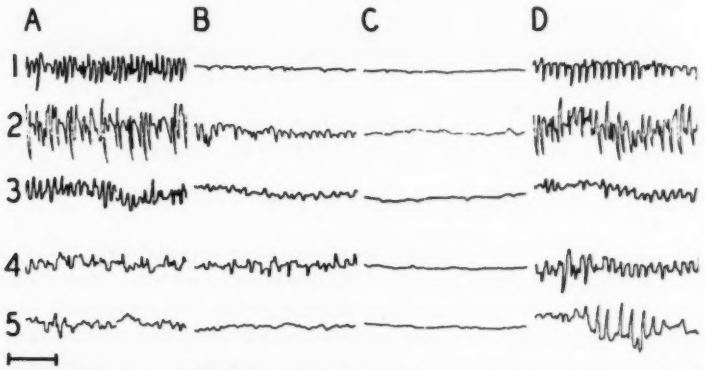

Fig. 2. Simultaneous effects of hyperventilation at several regions of the cerebral cortex. Stimulating electrodes on left suprasylvian gyrus, middle portion. Records 1, 2 and 3 from pairs of electrodes on the frontal, temporal and occipital regions of the left hemisphere respectively; 4 and 5 from the frontal and posterior parts of the right suprasylvian gyrus. A to D are strips showing the responses about 2 sec. after their beginning. Calibration: 0.5 sec.

A, normal response. B and C, after 3 and 5 min. of hyperventilation, respectively. D, 6 min. after the hyperventilation was stopped.

drop was followed after some irregular waves by a secondary slow progressive decrease. This striking change of blood pressure required some controls.

First the effect on the self-sustained responses of some other procedures that produce a decrease of blood pressure was tested, i.e., electrical stimulation of the peripheral ends of the vagus nerves (fig. 3 A) and obstruction of the venous return by compression of the heart (fig. 3 B). Under both of these conditions the cortical responses were markedly decreased.

In some demonstrative experiments after the self-sustained responses were clearly decreased by hyperventilation, a definite improvement was obtained by bringing the blood pressure back to normal by the procedure described in C; they became depressed again when the pressure was released.

The obvious mechanism suggested by these results was that the depression was caused by the ischemic condition of the brain. This factor was tested by clamping the intact carotid (the other one being previously occluded for blood

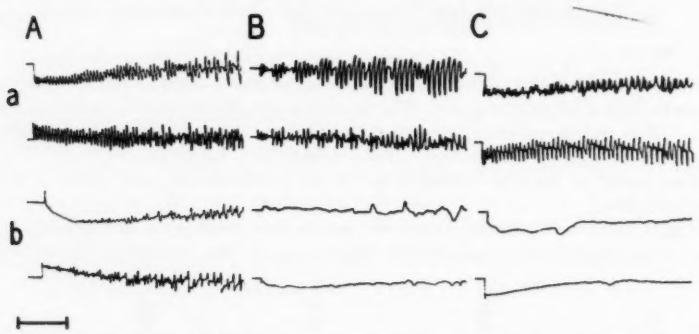

Fig. 3. Effect of decreased blood pressure and impaired cerebral circulation on the self-sustained responses of the cerebral cortex. The figure shows in each case the beginning of the response to cortical stimulation. Time calibration: 0.5 sec.

A. Before, *a*, and after, *b*, 40 sec. of stimulation of the peripheral end of the left vagus.

B. Before, *a*, and after, *b*, 1 min. of obstruction of the venous return by compression of the heart.

C. Before *a*, and after, *b*, 1 min. of occlusion of both carotid arteries.

pressure recording). The cortical responses were quickly reduced in spite of the reflex increase of blood pressure. Half a minute after the clamping, the responses were already reduced; 1 to 3 minutes of occlusion were enough to suppress any cortical response and even the spontaneous activity disappeared (fig. 3 C).

C. *Hyperventilation without changes of blood pressure.* In order to eliminate the circulatory factors, the fall of blood pressure that occurs during hyperventilation was avoided by simultaneous occlusion of the abdominal aorta. The marked increase of pressure that can be obtained by this procedure was adjusted to the desired value, as described under Methods (p. 335). Despite the unchanged blood pressure, acapnia resulting from hyperventilation decreased the cortical responses, but the effect took a longer time to become evident and it was less marked than the decrease when the blood pressure went down (fig. 1 B). Increases of blood pressure above normal values during normal breathing failed to produce any change of the responses.

D. *Hyperventilation without acapnia.* Hyperventilation without acapnia was obtained by the administration of air with 4 to 5 per cent CO_2. There was no decrease of the self-sustained responses if the blood pressure was kept normal, but some decrease when the blood pressure was not prevented from falling (fig. 4).

Hyperventilation with high concentrations of CO_2 (8 to 10 per cent) did not decrease cortical responses. Clamping of the aorta was then unnecessary to preserve a normal blood pressure, since after an initial and brief drop, the pressure recovered by itself to its previous value (fig. 4 C).

E. *The spontaneous activity of the cortex.* It was not the purpose of this study to consider systematically the changes of spontaneous activity during hyperventilation, and only incidental observations will be reported here. In general

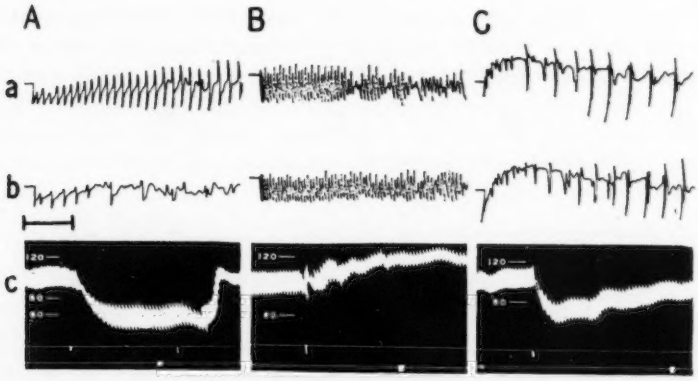

Fig. 4. Effect of hyperventilation without acapnia on the self-sustained response of the cerebral cortex. The electrical records show the beginning of the responses to cortical stimuli. The kymographic records show the blood-pressure changes during the periods of hyperventilation, as indicated by the upper signal. The lower signal marks cortical stimulations. Calibrations, for *a* and *b*: 0.5 sec.; for *c*: millimeters of mercury.

A. Before, *a*, and after, *b*, 1 min. of hyperventilation with 4 per cent CO_2.

B. As in A, but the fall of blood pressure was prevented by occlusion of the aorta. The record of Bb was taken after 5 min. of hyperventilation.

C. Before, *a*, and after, *b*, 3½ min. of hyperventilation with 8 per cent CO_2.

it may be said that the spontaneous activity presented changes in the same direction as those of the self-sustained responses. In other words, hyperventilation produced always a decrease of activity (fig. 5); this decrease was more striking when a fall of blood pressure accompanied the acapnia, but it could be seen also when that fall was prevented. This effect was found also in cats with mesencephalic transection in opposition to what Bremer (1935b) reported. There was no temporal correlation, however, between the changes in spontaneous activity and those in the tonic-clonic responses. Thus, the decrease of the latter responses occurred sometimes before any change of the spontaneous background was detectable; and sometimes the background spontaneous waves decreased first. We are unable to report the changes in pattern of the background activity, because they were not systematic in the short records taken.

By "decrease of activity" is meant that in general there were simultaneous falls of voltage and frequency which predominated over any occasional increase in the duration of some potential changes. Frequently the peculiar pattern in the cortical activity of an animal under barbituric anesthesia was maintained throughout an experiment and variations were observed only in the voltage and frequency of the individual waves of the "spindles."

Discussion. Hyperventilation decreases the self-sustained responses of the cerebral cortex. This effect was invariably observed in these experiments, as reported in section A. The decrease affected three features of the responses: the duration, and the voltage and frequency of the components. It is interesting to contrast these results with the ability of hyperventilation to induce attacks of "petit mal" in epileptic patients (Foerster, 1924).

The report of Brody and Dusser de Barenne (1932) that hyperventilation causes an increase of the motor responses to cortical stimulation seems to be in contradiction with the present observation. It is pertinent to point out, how-

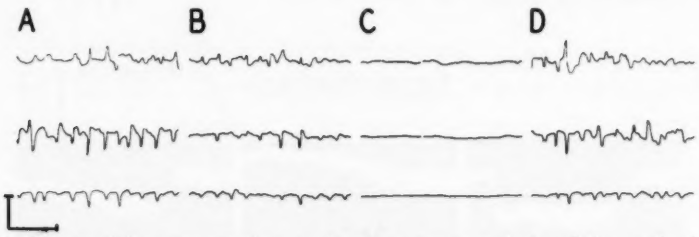

Fig. 5. Effects of hyperventilation on the spontaneous activity of the cerebral cortex of the cat. Electrodes on the frontal, middle and posterior portions of the suprasylvian gyrus. Calibrations: 0.5 sec. and 500 μv.

A. Normal record. B and C, after 1 and 3 min. of hyperventilation, respectively. D, 1½ min. after the hyperventilation was stopped.

ever, that Brody and Dusser de Barenne studied responses which were not the electrical potentials of the cortex but muscular contractions recorded by a Marey tambour. It is evident that in that procedure there are many steps and structures involved that may react to hyperventilation in different or even opposite ways. Striking among such possible effects is the increase of excitability of nerve and the change of accommodation that sometimes make "repetitive" the responses to single-threshold shocks (Rosenblueth and del Pozo, 1942). Brody and Dusser de Barenne reported also in the same paper some observations under strychnine in which hyperventilation or $NaHCO_3$ not only produced bigger responses but also a shortening of latency, epileptoid after-discharges, and spread to other muscular groups. These effects suggest a central action of hyperventilation, but the number of factors at play under those conditions makes an evaluation difficult.

Variable effects of hyperventilation on different structures may explain why, notwithstanding the remarkable action that acapnia has on the excitability and accommodation of nerve, a reflex may be decreased, as was the knee-jerk in the studies of Henderson (1910), or may be unchanged, as in four cats tested in our

series for the effect of hyperventilation on the flexor reflex. Decrease of hearing (Gellhorn and Spiesman, 1935), of visual intensity discrimination (Gellhorn, 1936) and several other depressant actions may be mentioned. The increase of visual sensitivity of the dark-adapted eye (Wald, Krieger and Goodman, 1942) further illustrates the variability of the effects of hyperventilation, depending on the structure considered.

The marked fall of blood pressure during hyperventilation showed an initial drop and a secondary progressive decrease. When the hyperventilation was carried out with gaseous mixtures containing over 4 per cent CO_2, the sharp initial drop persisted, but it was followed by a gradual recovery of the previous normal blood pressure. These results demonstrate that only the initial fall of blood pressure is due to the change in the intrathoracic pressure. The progressive secondary fall that takes place when ordinary air is used for hyperventilation is due to the acapnia induced (Dale and Evans, 1922).

Striking decreases both in self-sustained cortical responses and in spontaneous activity were observed in our experiments during decreases of blood pressure induced without changes in respiration (p. 338, fig. 3). Beecher, McDonough and Forbes (1938) found the same effect on spontaneous activity during falls of blood pressure produced by Cannon's method of cardiac tamponade (Cannon, 1923). Such remarkable decreases in cortical activity are probably due to cerebral ischemia (p. 338, fig. 3 C). The recovery from the ischemic effects was very slow in spite of the fact that the vertebral arteries were not occluded (see Stewart, Guthrie, Burns and Pike, 1906). Large as the effect of the fall of blood pressure is in decreasing the cortical activity, during hyperventilation the acapnia adds its independent effect, which was evident in the experiments under normal blood pressure (p. 338, fig. 1 B).

The spontaneous activity was not specially studied, but our incidental observations are in accord with other reports on the slow frequency of the components during hyperventilation (p. 339, fig. 5). Gibbs, Williams and Gibbs (1940), using the Grass analyzer, found that associated with low CO_2 tension in the jugular blood during hyperventilation there is an increase in the total energy and a shift toward the slow end of the frequency spectrum in the electroencephalogram. We did not notice any obvious increase of energy, and the occasional changes of pattern were not consistent enough to warrant detailed discussion.

It would be natural to suppose that the hyperventilation that we produced would cause an acapnia, but we have no direct information about the state of the cortex. Dusser de Barenne, Marshall, McCulloch and Nims (1938) have found changes in the local pH of the cortex correlated with cortical activity which were not always parallel with the pH of the blood. Furthermore, the vasoconstrictor effect of a reduction of CO_2 may oppose the loss of CO_2 from the cortex. Gibbs, Gibbs, Lennox and Nims (1942) present evidence that indicates the primary importance of the CO_2 tension of the brain in determining the electroencephalographic changes that occur during and after hyperventilation. Davis and Wallace (1942) had previously suggested that the changes might be the result of anoxia produced by the vasoconstriction. Since in our experiments neither

CO_2 tensions nor O_2 tensions were measured, any discussion about the mechanism underlying the changes that we observed in the cortical activity would be purely speculative.

SUMMARY

The effect of hyperventilation on the self-sustained responses of the cortex was studied in cats. Hyperventilation produced a decrease of the self-sustained responses. This decrease affected the duration of the response, and the voltage and frequency of the discharges (p. 336, figs. 1 and 2).

The decrease of the responses was observed in animals in which the blood pressure was kept unchanged during hyperventilation (p. 338, fig. 1 B).

Induced falls of blood pressure, with normal respiration, produced also marked decrease of the self-sustained responses (p. 338, figs. 3 A and B). Cerebral ischemia by bilateral carotid occlusion resulted in the same effect (p. 338, fig. 3 C).

Incidental observations were made on the effects of hyperventilation on the spontaneous activity of the cortex. Decreases in voltage and frequency were found during hyperventilation (p. 339, fig. 5).

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THE EFFECT OF CAFFEINE AND COFFEE EXTRACT ON THE ACTIVITY OF THE DIGESTIVE ENZYMES

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The results reported in the literature on the effect of coffee or its constituents on enzymic activity are meager. Sabalitschka and Schulze (1925) found caffeine to have no effect on malt amylase. Ohnishi (1928) reports activation of protease by caffeine. Heide and Schilf (1929) in experiments *in vivo* found that milk was more rapidly digested in the stomachs of rats when mixed with an infusion of coffee. Zlatarov and Popov (1937) conclude from the results of experiments in which the hydrogen ion concentrations of the substrates were not properly adjusted that caffeine has a slightly favorable influence on pepsin and trypsin but is without effect on lipase and ptyalin. In view of this lack of data, and because of the widespread belief that coffee interferes with digestion, it was thought that a careful and systematic investigation of the effect of caffeine and coffee extract on the activity of the digestive enzymes *in vitro* would be of value. The enzymes studied were those which act upon carbohydrates, proteins, and fats in the digestive tract, namely, ptyalin, pancreatic amylase, pepsin, trypsin, and pancreatic lipase.

EXPERIMENTAL PROCEDURES. The water used in making up the substrates upon which the enzymes were allowed to act and all solutions added to them was obtained by distilling ordinary distilled water from alkaline potassium permanganate. This distillate was acidified with phosphoric acid and again distilled.

The coffee used was a commercial brand purchased as the roasted bean and ground. It was stored in a glass stoppered bottle. The extract was prepared by adding 5 grams to about 100 cc. of boiling water and allowing it to stand five minutes with occasional stirring. It was then filtered through a coarse filter paper and the filtrate made up to exactly 100 cc. and used within half an hour. The caffeine was Merck's U.S.P. powder further purified by recrystallization. In these experiments both the caffeine and coffee were tested in two concentrations, namely, 20 and 40 mgm. of caffeine and 20 and 40 cc. of coffee extract per 100 cc. of substrate.

In all the digestion mixtures the hydrogen ion concentration was most carefully checked and adjusted so that any variation in the action of the enzyme could not be attributed to this factor. The measurements were made electrometrically with a Leeds and Northrup Universal pH Indicator, a glass electrode being used.

Amylolytic digestions. The amyloclastic materials studied were ptyalin in fresh filtered saliva, and a high grade commercial pancreatin. Two cubic centimeters of the filtered saliva were made up to 50 cc. and 1.5 cc. of this diluted material was added to each digestion mixture. In the case of the pancreatin 1 cc. of a filtered solution containing 1 mgm. per cubic centimeter was used.

The activities of the salivary and pancreatic amylases were measured by determining the amount of reducing sugar formed. The method used was that of Sherman and co-workers (1921). The enzyme was allowed to act for 30 minutes at 40° C. on 100 cc. of a 1 per cent dispersion of soluble starch to which had been added as activators for the amylase 5 cc. of M sodium chloride and 2.5 cc. of 0.02 M disodium hydrogen phosphate. The pH of the substrate was in all tests adjusted to 7.0 by means of 0.01 M sodium hydroxide. At the end of half an hour the action of the enzyme was stopped by adding to the digestion flasks 50 cc. of Fehling's solution. These were immersed for 15 minutes in boiling water and the weight of cuprous oxide formed by the reducing sugar in this length of time was taken as a measure of the rate of digestion. Blanks were carried out under the same conditions but without added enzyme to determine the reducing action of the caffeine and of the coffee extract. In the case of the coffee extract the reducing action was found to be appreciable, so in all the results reported for coffee this value has been substracted. Caffeine showed no reducing action.

Proteolytic digestions. The protein splitting enzymes studied were high grade commercial pepsin and trypsin. One cubic centimeter of a 1 per cent solution of pepsin in 0.05 M hydrochloric acid was used for each digestion. The same amount of trypsin dissolved in water was used in each test with this enzyme. A substrate of 1 per cent casein (according to Hammarsten) was used. In the pepsin experiments 0.5 gram of casein was dispersed in 50 cc. of 0.05 M HCl, or in 50 cc. of the acid extract of coffee in the digestions in which the effect of coffee was studied, by warming to between 40° C. and 50° C. In the preparation of the caffeine solution and the coffee extract 0.05 M HCl was also used instead of water. The pH of the digestions mixtures was adjusted to 1.34. The pepsin was allowed to act on 50 cc. of the substrate for 1 hour at 40° at the end of which time the undigested casein was precipitated by the addition of 12.5 cc. of 20 per cent sodium sulphate. After standing overnight the mixture was filtered and the filtrate made up to 100 cc. The nitrogen in a 10 cc. portion of this filtrate was determined by a semimicro modification of the Kjeldahl method.

The same general procedure was carried out for trypsin digestions except that the casein was dispersed in a dilute sodium hydroxide solution. The pH of the substrate was 8.22. To the mixtures to which caffeine or coffee was added, sufficient sodium hydroxide solution was also added to adjust the pH to this same value.

Semimicro determinations of the nitrogen in the added caffeine and coffee extract were made, and this amount was substracted from the results obtained in the proteolytic digestions when these substances were present.

Lipolytic digestions. In these experiments the influence of caffeine and coffee on the digestion of olive oil by steapsin, the fat splitting enzyme secreted by the pancreas, was determined by the following procedure: 2 cc. of neutralized olive oil were measured into a 125 cc. Erlenmeyer flask. To this was added 1 cc. of 6 per cent bile salt (sodium taurocholate), 1 cc. of caffeine solution (or neutral coffee extract, or water in case of the control), and 1 cc. of steapsin solution. The flasks were shaken vigorously and placed in a bath at 40° C. for 2½ hours. At

frequent intervals during the digestion the shaking was repeated. At the end of the digestion period, 20 cc. of isopropyl alcohol were added and the amount of fatty acids formed was determined by titration with 0.05 M sodium hydroxide, bromthymol blue being used as indicator. Since all the digestion mixtures in a comparative set of experiments could not be titrated at the same time, it was necessary, in order to stop the enzymic action, to keep the flasks immersed in crushed ice during the interval between removal from the bath and titration.

In preliminary experiments carried out to determine the best conditions for the estimation of the fatty acids formed, it was found that a more satisfactory end point was obtained when isoprophyl alcohol was used instead of the ethyl alcohol usually employed in this determination. Because of the color of the coffee it was also found in the experiments with this material that phenolphthalein was worthless as an indicator, but that bromthymol blue gave a readily discernible and definite end point.

In the lipolytic experiments the volume of the substrate was 5 cc. so the amounts of caffeine added in the two series of experiments were 1 mgm. and 2

TABLE 1

Effect of coffee extract on salivary and pancreatic amylases and pancreatic lipase

COFFEE EXTRACT PER 100 CC. OF SUBSTRATE	SALIVARY AMYLASE		PANCREATIC AMYLASE		PANCREATIC LIPASE	
	Cu_2O		Cu_2O		0.05 M NaOH equiv. to acids formed	
cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*cc.*	*cc.*
None	296		246		23.1	
20	318		273		20.7	
None		339		195		21.3
40		353		218		13.2

mgm. which would be equivalent to 20 mgm. and 40 mgm. per 100 cc., the same concentrations used in the amylolytic and proteolytic digestions. Likewise the volumes of coffee extract added were equivalent to 20 cc. and 40 cc. per 100 cc. of digestion mixture.

RESULTS Caffeine when present in concentrations of 20 mgm. and 40 mgm. per 100 cc. of substrate showed absolutely no effect on the activity of any of the enzymes studied, namely, salivary and pancreatic amylases, pepsin, trypsin, and pancreatic lipase.

The coffee extract likewise was without effect on the proteolytic enzymes, pepsin and trypsin. It is recognized, however, that the concentration of hydrochloric acid necessary for the activity of pepsin doubtless altered the nature of the coffee extract, and that in our experiments, therefore, it was not the effect of the original constituents of the coffee that was determined but of the products formed by the acid treatment. But since this acidity is comparable to that of the gastric juice, in which medium pepsin acts *in vivo*, it may be assumed that coffee undergoes similar changes in the stomach and would produce a similar effect on the action of pepsin *in vivo*. The experiments showed, however,

a decided increase in the rate of digestion by both salivary and pancreatic amylase when coffee extract was present. This is shown in table 1. Since this favorable influence is not due to the caffeine present, it must be attributed to some other of the many constituents of coffee.

All determinations the results of which are compared were carried out in duplicate and at the same time under exactly the same conditions. Duplicate determinations showed an experimental error of less than 0.5 per cent so the variations recorded below, while not large, are certainly significant. The results given in the table are averages of several experiments. Table 1 also shows the results obtained with the pancreatic lipase. The activity of this enzyme was retarded when coffee extract was added to the digestion mixture, this effect being quite large when 40 cc. of the extract per 100 cc. of substrate were present. This retardation is doubtless due, at least in part, to the fact observed in these experiments, that the coffee infusion markedly decreased the stability of the olive oil emulsion used as substrate. This would obviously produce an unfavorable condition for the interaction of enzyme and fat molecules.

SUMMARY

1. Caffeine in concentrations of 20 mgm. and 40 mgm. per 100 cc. of substrate has no effect *in vitro* on the saccharogenic action of salivary and pancreatic amylases; nor does it affect the digestion of casein by pepsin and trypsin, or of olive oil by pancreatic lipase.

2. Coffee extract in the two concentrations studied does not affect the digestion *in vitro* of casein by pepsin or trypsin.

3. Coffee extract in the two concentrations studied increases the rate of digestion of starch by the salivary and pancreatic amylases.

4. Coffee extract in the two concentrations studied retards the digestion of olive oil by pancreatic lipase.

5. An improvement in the lipolytic method was made by using isopropyl alcohol instead of ethyl alcohol, and bromthymol blue as indicator instead of phenolphthalein in the procedure for the titration of the fatty acids formed.

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THE RÔLE OF THE BRAIN STEM IN ARTERIAL HYPERTENSION SUBSEQUENT TO INTRACRANIAL HYPERTENSION

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Cushing (1902) described the arterial hypertension which occurs immediately upon the experimental elevation of intracranial pressure to a level above that of the diastolic blood pressure. He further demonstrated that section of the vagus had no effect upon this phenomenon but that either section of the cervical spinal cord or the introduction of cocaine into the fourth ventricle obviated the blood pressure response. From these findings he concluded that the blood pressure regulating mechanism is a function of the medullary vasomotor centers and is released by the anemia induced by the elevation of the intracranial pressure to a level above that of the blood pressure.

Subsequently it was learned that pressor responses could be obtained by stimulation of certain portions of the central nervous system rostral to the pons. Ranson and Magoun (1939) in their monograph on the hypothalamus reviewed the experiments of others and added their own observations regarding the areas of the hypothalamus from which pressor responses could be elicited by stimulation. Hoff and Green (1936) and Crouch and Thompson (1936) described pressor responses on stimulation of discrete portions of the cerebral cortex. The possibility therefore arises that the pressor responses to increased intracranial pressure might originate, not in the medullary centers, but rather in these higher centers and thus the medullary centers might represent the final synapse, a position analogous to that of the anterior horn cell in the motor system.

While it is thus conceivable that the mechanism might originate as a supra-pontine one, the possibility also arises that the origin of the pressor response might be in the medullary centers but the efferent arc extends rostrally through the brain stem. Chang and his collaborators (1937, 1940) and Sattler (1940) described a vago-supra-optic-posterior pituitary pressor mechanism upon stimulation of the central end of the sectioned vagus nerve.

The introduction of cocaine into the fourth ventricle could then obviate the pressor response to increased intracranial pressure in any one of three ways; the depression of the medullary centers, the interference with either ascending or descending pathways between these structures and pressor areas situated more rostrally, or by diffusion into the third ventricle and directly affecting the hypothalamic nuclei.

It therefore remains to be determined whether the medullary vasomotor centers are those primarily concerned in the blood pressure response to increased intracranial pressure and whether the connections—either ascending or descend-

ing—of these centers to higher pressor areas are necessary. To determine this studies were made of the blood pressure responses to increased intracranial pressure before and after decerebration and destruction of the medulla.

METHOD. Nine cats were studied. Surgical anesthesia was induced by intraperitoneal injection of 10 per cent sodium amytal (0.6 cc. per kgm.). A tracheal cannula was inserted, a midline scalp incision made and skin, galea, and muscles reflected. A trephination ($1\frac{1}{2}$ cm. in diameter) was performed over the right parieto-occipital area as far inferiorly and posteriorly as was possible while still avoiding the bony tentorium. Following radial incision and reflection of the dura, a fitted, threaded, metal cannula was inserted into the trephination and attached to a mercury manometer and writer and a pressure bottle containing physiological saline. Blood pressure recordings were obtained from the femoral artery by means of a mercury manometer and writer, the anti-coagulant being citrate. In all experiments, the animals were placed on artificial respiration with the onset of respiratory failure during the first intracranial pressure rise, and this artificial respiration was continued for the duration of the observations.

An initial record was taken of the blood pressure response to raised intracranial pressure. The cranial cannula was then removed and the animal was decerebrated. This was performed by introducing a blunt probe into the trephination below the occipital lobe, following the bony tentorium and then cutting the brain stem on the same plane as the tentorium. This resulted in a section either between the inferior and superior colliculi or through the superior colliculi. Cerebral edema occurred at this point but the trephination, by serving the purpose of a decompression, allowed this to recede. The cannula was then replaced and the pressure changes studied. The medulla was destroyed by maceration by means of a blunt probe introduced through the trephination.

The animals were sacrificed by intra-aortic injections of saline followed by 10 per cent formalin. In this manner the brain stem was fixed in situ and the damage wrought by maceration was more accurately determined.

RESULTS. Following decerebration the blood pressure response to an increased intracranial pressure was not only obtainable (fig. 1) but when compared with the results before decerebration was augmented both as to height and duration. Whereas prior to decerebration the blood pressure level exceeded the intracranial pressure level by a customary range of 15–40 mm. Hg the difference after decerebration was in the neighborhood of 30–90 mm. Hg.

The persistence of the blood pressure response after decerebration occurred in eight of the nine animals. In the first experiment attempted the cannula was replaced immediately after decerebration and without allowing the cerebral edema to subside. A spontaneous blood pressure rise occurred when the cannula was replaced. Subsequent intracranial injection of saline produced no further blood pressure response. In the remaining eight animals time was allowed for subsidence of the cerebral edema and in all instances adequate blood pressure responses were obtainable.

Maceration of the medulla to the level of the superior border of the inferior

olive resulted in the abolition of the blood pressure response (fig. 2). When the destruction included only areas rostral to this level the blood pressure response was obtainable. These results following maceration were obtained in all of the three animals upon whom this procedure was carried out. Whenever the blood pressure response had been obviated in the above manner it was still possible to produce an adequate blood pressure rise (to 200–220 mm. Hg) by the intravenous injection of adrenalin, thus indicating that peripheral mechanisms for the production of hypertension were still available.

Discussion. The conclusions drawn from these results are that the vasomotor centers essential for the production of the vascular hypertension subsequent to intracranial hypertension extend no farther rostrally than the level of

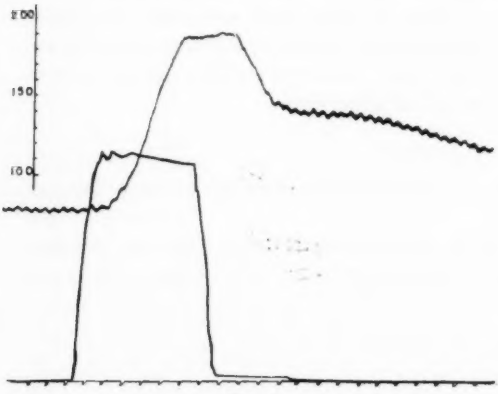

Fig. 1

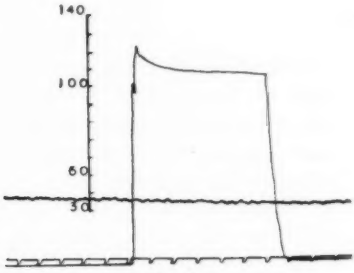

Fig. 2

Fig. 1. Cat under amytal anesthesia and decerebrated. Upper tracing, blood pressure; lower tracing, intracranial pressure. Numbered scale in mm. Hg. Time interval 5 sec. Demonstrates, following decerebration, an adequate rise in blood pressure level upon elevation of the intracranial pressure level.

Fig. 2. Same cat under amytal, decerebrated and brain stem macerated to level of the superior border of the inferior olive. Scale values and time intervals as in figure 1. Demonstrates no rise in blood pressure level upon increasing the intracranial pressure level.

the superior border of the inferior olive and that the neuronal connections—whether afferent or efferent—of these centers with higher vasomotor centers are non-essential. Indeed the blood pressure rise is higher and the hypertension is longer maintained when these connections are severed.

These results are not inconsistent with the findings of Denny-Brown and Russell (1941) who noted that in concussing decerebrate preparations blood pressure rises occurred. Their experiments are not quite comparable to the ones here discussed because of the obviously more complicated situation in concussion, following which, as they showed, elevation of the intracranial pressure occurs only under certain conditions.

Dock and his collaborators (1942) studying renal hypertensive and controlled dogs found that crushing the brain stem rostral to the pons produced a

fall in blood pressure with a tendency to regain previous levels and still maintaining a distinct difference in levels between the hypertensive and controlled groups. Destruction of the cord, medulla and pons, however, yielded a sharp fall in blood pressure in both groups and without a significant difference in the blood pressure levels of hypertensive and control animals.

The decerebration studies reported here show that anatomical segregation of the medulla and pons from neural structures rostral to them does not obviate the increase in blood pressure due to intracranial hypertension. This indicates that neither anemia of the cortex and hypothalamus nor involvement of the vago-supra-optic mechanism can play an active part in this response since it persists after both the ascending and descending pathways have been severed.

Destruction of the medulla obviates the blood pressure response to increased intracranial pressure. Therefore, it may be concluded that the vasomotor centers essential for arterial hypertension subsequent to intracranial hypertension are situated in the medulla, and that the efferent pathways do not progress further rostrally in the brain stem than this level.

CONCLUSIONS

1. The blood pressure response to increased intracranial pressure depends upon the integrity of the medulla.

2. Neural structures rostral to the medulla are not necessary for this response.

3. Anatomical separation of the medulla and pons from higher structures enhances the response.

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OBSERVATIONS ON THE CIRCULATION IN THE HIND LIMBS OF A DOG TEN YEARS FOLLOWING LEFT LUMBAR SYMPATHETIC GANGLIONECTOMY

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We have reported (1) simultaneous observations on the blood flow in the femoral arteries of denervated and innervated limbs. Control observations were made before and at various intervals of time following denervation by complete sympathetic ganglionectomy from the first or second lumbar ganglion to the end of the chain. It was found repeatedly that the blood flow on the denervated side was about twice that on the innervated side provided the operative procedures for applying the thermostromuhr units were done with the animal under local anesthesia. Under general anesthesia with ether the difference of blood flow was not apparent, the flow in the two sides becoming practically the same.

We were interested to determine how long this difference of blood flow would persist. In a series of experiments observations were made at different time intervals following lumbar sympathetic ganglionectomy. The longest interval was two years and ten months after sympathectomy and since the disparity of the flow of the two femoral arteries still persisted it seemed reasonable to conclude that the difference of blood flow in the hind limbs resulting from unilateral sympathetic ganglionectomy was permanent in the dog.

The present communication is concerned with observations which have indicated that the conclusion just mentioned is open to question. We shall present evidence obtained from the study of one animal indicating that, if a sufficient time after operation has elapsed, the increased flow in the femoral artery following sympathectomy may be reduced to approximately that in the control or innervated side.

METHODS AND RESULTS. As in other studies on blood flow the thermostromuhr was used. The operation for applying the units to the femoral arteries was done with infiltration anesthesia using procaine. The blood flow in both femoral arteries was observed simultaneously. Lumbar sympathetic ganglionectomy was done on the left side using sterile technic and with the animal under general anesthesia.

We desired to make observations on some of the animals of our original series after several years had elapsed. However, since we became interested in other investigations, further observations on the sympathectomized dogs were postponed and more than nine years had passed before we returned to the problem. By that time only one of the original group of dogs remained. Examination of data obtained on this animal indicated that in a control observation made before

sympathectomy the flows in the right and left femoral arteries were respectively 108 and 77 cc. each minute. Fifteen days after sympathectomy the blood flow on the right and left sides was respectively 99 and 297 cc. each minute. Then again nearly eleven months after the operation the flow on the right side was 188 cc. and that on the left or denervated side was 377 cc. each minute. When the blood flow in the femoral arteries of this remaining dog was measured more than nine years after sympathectomy the flow in the right femoral artery was 112 cc. and that in the left was 125 cc. each minute (table 1).

In seeking an explanation of this unexpected result it was decided to observe the effects of intravenous injections of epinephrine and histamine. It was found that the flow in the femoral artery of the innervated limb was practically unaffected by the doses of epinephrine used but the sympathectomized side showed a maximal response since the flow appeared to be completely shut off. Following injections of effective doses of histamine the results on the two sides were again

TABLE 1

Blood flow in femoral arteries of trained dog before and after left lumbar sympathetic ganglionectomy

OBSERVATION	FLOW	
	Right femoral	Left femoral
	cc. per min.	*cc. per min.*
Before sympathectomy I. A. P.*	108	77
15 days after sympathectomy I. A. P.	99	297
10 months, 25 days after sympathectomy I. A. P.	188	377
9 years, 2 months, 1 day after sympathectomy I. A. P.	112	125
Same except under pentobarbital sodium anesthesia	131	140
10 years, 2 months, 22 days after sympathectomy I. A. P.	91	110

* I. A. P. = Infiltration anesthesia with procaine.

very different and quite the reverse of what followed injections of epinephrine. The flow on the denervated side was only slightly affected whereas that on the innervated side was markedly increased (table 2).

A plethysmograph was later placed on each hind foot, after which the same drugs were given. The changes of the volume of the foot reflected what had occurred when the blood flow was recorded. There was a transient decrease of the volume of the innervated foot with small doses of epinephrine but a very prolonged decrease of the volume of the denervated foot. When histamine was given the denervated foot showed only a slight increase while the innervated foot increased markedly in volume. The difference of the response of the two limbs to vasoconstrictor and vasodilator drugs raised the question of the possibility that a change had occurred in the walls of the blood vessels of the denervated side. We conceived of the vessels as being inclosed by thick connective tissue which might prevent dilatation but yet permit contraction. At the suggestion of Doctor Barker a toe was removed from each hind foot of this animal and the tissues were studied microscopically. Sections of the tissues of the two toes

were placed alternately on slides in a certain sequence. The observer was unaware of the identity of the sections. He was asked to determine whether there was any difference that he could detect between the vessels of the successive sections. Several of our colleagues reported that the arterioles seen in certain

TABLE 2

Effect of epinephrine and histamine on blood flow in right and left femoral arteries nine years, two months and one day after left lumbar sympathetic ganglionectomy

DRUG	RIGHT ARTERY FLOW	LEFT ARTERY FLOW	ANESTHETIC
Epinephrine, 0.05 cc. 1:1,000 in 15 seconds	Slight increase, slight decrease	Zero flow for 1 minute	Local infiltration anesthesia with procaine hydrochloride
	Slight increase, slight decrease, return to control	Zero flow for 1 minute	
Histamine, 0.01 mgm. per kgm.	240 per cent increase	90 per cent increase	
	74 per cent increase	3 per cent increase	Pentobarbital sodium anesthesia
Epinephrine, 0.05 cc. 1:1,000 in 15 seconds	Slight increase, slight decrease	Zero flow for 2 minutes	

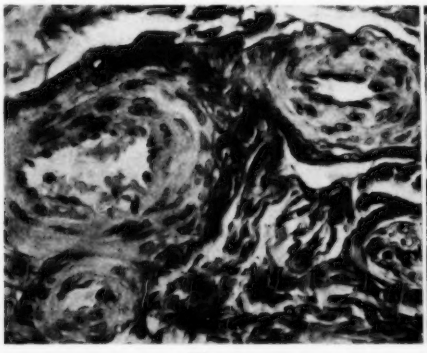

Fig. 1

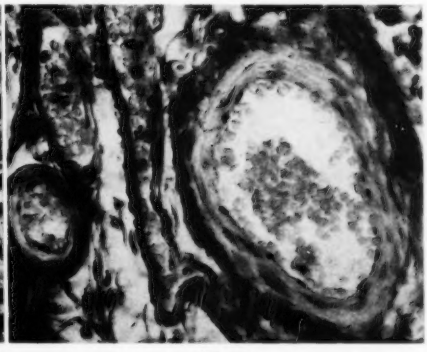

Fig. 2

Fig. 1. Tissue from a toe taken from the left foot of a dog more than nine years after sympathetic ganglionectomy on the left side.

Fig. 2. Same as figure 1 except the tissue was from the right or control side.

of the sections were markedly thickened as compared with what was seen in the other sections. It was found that hypertrophy of the arterioles was present on the denervated side and was confined to the media or muscularis coat of the vessels (fig. 1). The vessels of the control side (fig. 2) did not show hypertrophy.

A year later, or ten years, two months and twenty-two days after sympathetic ganglionectomy, the flow in the two hind limbs was again observed. At that

time the flow in the femoral artery of the innervated limb was 91 cc. and that in the denervated limb was 110 cc. each minute. The effects of injections of epinephrine and histamine were the same as already described. Under pentobarbital sodium anesthesia the effect of epinephrine and histamine on the blood flow in the two limbs was observed with results similar to those described before in this report.

In addition a small artery less than a millimeter in diameter was exposed in both legs just below the knee. The behavior of these arteries was observed before and after administration of epinephrine. The response, a marked constriction of the artery on the denervated side, was much greater than that of the control side. Both arteries were then cut so that a vigorous stream of blood issued forth. After epinephrine was given, the vessel on the denervated side constricted sufficiently to shut off the blood flow whereas the vessel on the control or innervated side continued to bleed profusely. That the lack of flow on the denervated side was not the result of formation of a clot was demonstrated by the resumption of profuse bleeding as soon as the effect of the injected epinephrine was dissipated.

Thus it appears that both the arterioles and the small arteries of the denervated side were more sensitive to epinephrine than those of the innervated side. Observations were made on the animal under local and under general anesthesia with pentobarbital sodium until death. All observations were confirmatory of those made a year previously as were the findings on additional sections made from tissues removed from the two legs. A difference of the blood vessels of the two sides was not apparent above the knee of the animal. At necropsy a careful search of the left lumbar tissues was made but there was no gross evidence of sympathetic ganglia or sympathetic nerves being present.

Comment. At the outset it must be emphasized that all the observations recorded in this report were made on a single animal but at the same time it should be pointed out that a better controlled experiment is difficult to devise. One hind leg of a given animal should offer the ideal control on the other hind leg. The number of variables introduced should be minimal. Furthermore it is not frequently that we are permitted observations on an experimental animal over so long a period of years. Therefore we may be excused for having discussed the findings on a single animal at considerable length.

The question at once arises as to the cause of the hypertrophy of the walls of the arterioles. Two possible explanations have been considered. 1. The hypersensitivity of the vessels of the denervated limb was sufficiently great to suggest the possibility of a vigorous response of these vessels to the animal's own secreted epinephrine, which over a period of years might result in a work hypertrophy of the muscles of the vessels. 2. It is common knowledge that sympathectomy impairs the tone of blood vessels. Consequently the vascular musculature is subjected to stretching which, if resisted over long periods of time, might result in muscular hypertrophy in the walls of the involved vessels.

Quite obviously the answer as to what brought about the difference in the small vessels of the two limbs must await the results of more experiments.

SUMMARY AND CONCLUSIONS

Observations have been made on the blood flow in the femoral arteries of a dog at intervals over a period of ten years and two months. Blood flow was measured in both femoral arteries simultaneously under infiltration anesthesia before sympathetic ganglionectomy and fifteen days; ten months, twenty-five days; nine years, two months and ten years, two months after sympathectomy. Whereas the flow was twice as great in the left or sympathectomized limb as it was in the right or control limb fifteen days and also ten months and twenty-five days after sympathectomy, the flow was almost the same in the two limbs nine and also ten years after sympathectomy.

That the vessels of the sympathectomized leg were profoundly hypersensitive to epinephrine more than nine and ten years following sympathectomy was shown by a small injection of epinephrine which caused such a marked constriction of the vessels of the sympathectomized side that the flow was zero for at least two minutes. The flow on the control side taken at the same time was only slightly altered.

Simultaneous plethysmographic records of the two hind feet further confirmed the observations on blood flow since there was a transient decrease of the volume of the innervated foot but a very prolonged decrease of that of the denervated foot in response to small doses of epinephrine.

Histologic examination of a toe from each hind foot showed that the arterioles of the left or sympathectomized foot had undergone hypertrophy which was confined to the muscularis coat of the vessels. The vessels of the control side did not show hypertrophy.

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THE EFFECT OF SODIUM BICARBONATE ON GASTRIC SECRETION[1]

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The effect on gastric secretion of $NaHCO_3$ administered by mouth has been controversial since Claude Bernard (1) first observed that it stimulated secretion. The majority of comparatively recent clinical reports give the impression that it induces an increase in acid secretion following the initial period of neutralization. Thus, Spencer et al. (2) found by fractional gastric analysis that 0.65 to 5.0 per cent solutions of $NaHCO_3$ produced higher curves of acidity than were obtained in controls. Crohn (3) reported that 2 grams of $NaHCO_3$ given after a test meal increased the acidity shown by fractional analysis. Lockwood and Chamberlin (4) noted that the inclusion of 4 grams of $NaHCO_3$ in the test meal raised the acidity in 50 per cent of their cases. These reports are largely the basis for the concept of a "rebound" or "secondary" secretion of acid which is frequently mentioned in current literature.

The nature of this "rebound" is not clear. Fractional analysis measures *concentration* of acid. Without minimizing the importance of acid concentration, we raise the question of whether the "rebound" acid secretion is an active stimulation or simply a passive rise in acid concentration. For example, it is known that $NaHCO_3$ decreases the emptying time of the stomach (5). In view of this and the fact that parietal cell secretion is constantly high in acid concentration, the increase noted by fractional analysis may be the result of diminished dilution of secretion which enters the stomach when it is more nearly empty.

The answer to the above question thus depends primarily on a comparison of the *amounts* of acid secreted during an entire digestive period *a*, under control conditions, and *b*, under conditions in which the "rebound" is elicited. Animal experimentation is necessary to obtain such data. The nearest approach to this type of data in the literature is Boyd's report (6). Two Pavlov pouch dogs were fed $NaHCO_3$ daily for more than 2 months. The mean rate of secretion and the mean acidity of the pouch collections were increased during the $2\frac{1}{2}$ hours immediately following the test meal. However, several factors complicate interpretation of these data: *a*, the daily period of secretion observed was relatively short; *b*, an appraisal of the effect of the intentional variation (inclusion of table scraps, etc.) in the daily meal, which served as the test meal, is difficult; *c*, the

[1] A preliminary report was presented before the American Society for Pharmacology and Experimental Therapeutics, Boston, 1942. Federation Proceedings **1:** 141, 1942.

[2] On leave in the medical service of the U. S. Army.

average data reported include days on which gastro-intestinal upsets (diarrhea, vomiting) occurred. Therefore, in the absence of sufficient data for an adequate evaluation, we have sought to determine the effect of $NaHCO_3$ on gastric secretion by use of the Cope pouch dog.

EXPERIMENTAL. This is apparently the first report of the experimental use of the Cope gastric pouch dog (7) and, therefore, the procedures developed in this laboratory and used in these experiments will be described in detail. A Cope pouch is made from most of the anterior wall of the body of the stomach by a two-stage operation which leaves the vagal nerve supply intact. Unlike most pouches, the stoma lies high on the dog's flank and no collection vessels, bandages, or restraints are required, since the pouch hangs dependent within the abdomen and serves as its own collection vessel. However, these conditions require an antacid in the pouch to prevent peptic ulceration. Measurement of the amounts of chloride, base, acid, and of the volume secreted during any interval desired may be made by using the technic described below, which permits continuous 24 hour study day after day.

The *control routine* employed was as follows: The pouch was emptied with a catheter and syringe at 8 and 11 a.m. and at 2 and 5 p.m. The test meal was fed immediately after the 8 a.m. collection. The three daytime collections following the meal, and the collection at 8 a.m. the following morning (night secretion) constituted a 24 hour collection. After each emptying a measured amount of aluminum hydroxide gel of known composition was placed in the pouch. Water was allowed ad libitum day and night. At the end of each week an arterial blood sample was taken before feeding the meal which included, on that day, one tablespoonful of brewer's yeast. Red and white cell counts, cell volume (8), O_2 and CO_2 capacity of whole blood (9), and plasma chloride (10) were determined.

The *test meal* given at 8 a.m. served not only as the stimulus of secretory activity, but also as a vehicle for the return of electrolytes lost via the pouch, and as an adequate diet. A weighed amount of a commercial dog food[3], sufficient to maintain weight and normal activity, was mixed daily at 4 p.m. with 50 cc. of 1 N HCl and an adequate amount of NaCl. The amount of the latter was determined by following the blood chloride and CO_2 capacity, after which it was kept constant from day to day. The meal buffered the HCl and was more palatable than when all necessary chloride was returned as NaCl. Each meal was consumed within 10 minutes and all animals had voracious appetites.

The *pouch antacid* was aluminum hydroxide gel[4] diluted 1:1 by volume with water. Individual animals required 25 to 50 cc. of this dilution to neutralize the acid secreted during the collection period. Aluminum hydroxide gel was used because: *a*, it is more efficient than $NaHCO_3$ in preventing ulceration of the Cope pouch; *b*, it maintains a pH within the pouch nearer the reaction of normal gastric contents; *c*, it is not absorbed, and *d*, it does not interfere with the electrolytic determination of base.

[3] Bovex (Old Trusty Dog Food Co., Needham Heights, Mass.).

[4] Amphojel, generously supplied by John Wyeth and Bro., Inc., Philadelphia, Pa.

Each pouch collection was filtered through a coarse fritted glass filter and the filtrate analyzed in duplicate for chloride (10) and base (11). Similar determinations were made on each lot of diluted aluminum hydroxide gel. Control studies showed that no appreciable error was introduced by filtration or by the presence of aluminum in the filtrate. Quantitative determinations (12) showed only negligible traces of aluminum in the cathode vessel in the electrolytic determination of base.

The volume of the original pouch collection in liters multiplied by the concentrations of chloride and base in milliequivalents per liter gave the amounts in milliequivalents of these constituents in the collection. From these values were subtracted the amounts of chloride and base initially present in the antacid to give the total chloride and total base of the *pouch secretion*. The amount of free acid secretion was estimated by subtracting the secretion total base from the

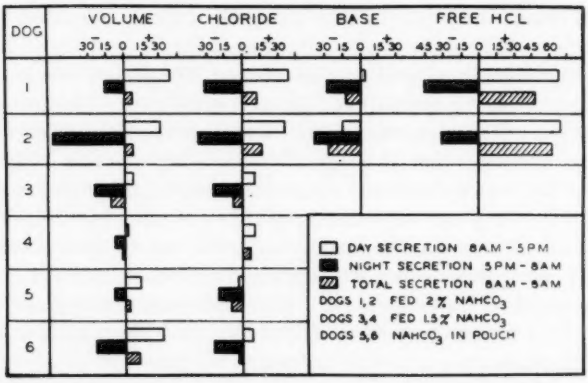

Fig. 1. The average per cent of change from controls in secretion of the Cope pouch when $NaHCO_3$ is fed by stomach tube, or when 4 per cent $NaHCO_3$ is placed in the pouch.

secretion total chloride. The *secretion volume* was obtained by subtracting the volume of antacid placed in the pouch from the volume of *pouch collection*.

Five Cope pouch dogs accustomed to the stomach tube were used. The first series of experiments consisted of volume and total chloride determinations on the pouch collection from dogs 3 and 4 on control routine for 30 days, with the exception that from the 7th to 18th day each dog received by stomach tube 50 cc. of 1.5 per cent $NaHCO_3$ solution daily at 9 and 11 a.m. and 2 p.m.

In a second series, dogs 1 and 2 each received 50 cc. of 2 per cent $NaHCO_3$ solution daily at 9 and 11 a.m. and 2 p.m. from Monday morning through Friday afternoon. A week of control and a week of $NaHCO_3$ feeding were alternated twice, giving 4 consecutive weeks of study. As a further control, the effect of administering 50 cc. of tap water, instead of the $NaHCO_3$ solution, was determined by a fifth week of observation. Complete analyses were made on the collections of this series.

The effect of $NaHCO_3$ in direct contact with the *pouch* mucosa was observed,

TABLE 1

*Effect of feeding sodium bicarbonate on gastric secretion in the Cope pouch**

TEST WEEK	COLLECTION PERIODS	SECRETION VOLUME	TOTAL CHLORIDE AMT. SECRETED	TOTAL BASE AMT. SECRETED	FREE HCl AMT. SECRETED
		cc.	*m.eq.*	*m.eq.*	*m.eq.*
Dog 1 (8 mos. post-operative)					
2/1–2/7/42 control	Day	152	29.6	13.0	16.6
	Night	91	18.0	14.4	3.6
	24 hours	243	47.6	27.4	20.2
2/8–2/14/42, fed $NaHCO_3$	Day	211	42.8	12.7	30.1
	Nights	30	10.3	8 6	1.7
	24 hours	241	53.1	21.3	31.8
2/15–2/21/42, control	Day	159	31.0	14.4	16.6
	Night	83	17.8	14.1	3.7
	24 hours	242	48.8	28.5	20.3
2/22–2/28/42, fed $NaHCO_3$	Day	220	40.6	16.3	24.3
	Nights	57	14.1	12.2	1.9
	24 hours	277	54.7	28.5	26.2
3/23–3/28/42, fed H_2O	Day	187	33.8	15.0	18.8
	Night	114	22.1	18.5	3.6
	24 hours	301	55.9	33.5	22.4
Dog 2 (7 mos. post-operative)					
2/1–2/7/42, control	Day	90	18.8	7.6	11.2
	Night	54	11.2	9.5	1.7
	24 hours	144	30.0	17.1	12.9
2/8–2/14/42, fed $NaHCO_3$	Day	112	25.0	6.4	18.6
	Night	14	5.4	4.6	0.8
	24 hours	126	30.4	11.0	19.4
2/15–2/21/42, control	Day	103	20.5	7.5	13.0
	Night	11	4.6	4.0	0.6
	24 hours	114	25.1	11.5	13 6
2/22–2/28/42, fed $NaHCO_3$	Day	139	28.4	6.7	21.7
	Night	11	4.6	4.4	0.2
	24 hours	150	33.0	11.1	21.9
3/23–3/28/42, fed H_2O	Day	114	21.3	6.9	14.4
	Night	32	7.0	6.2	0.8
	24 hours	146	28.3	13.1	15.2

* Each figure is the average of three values obtained on three consecutive days during the week indicated.

in a third series, by volume and total chloride determinations on the collections from dogs 5 and 6 (dog 5 was no. 3 of the first series). After a week of control

with aluminum hydroxide gel in the pouch, 3 weeks of study were carried out in which 4 per cent $NaHCO_3$ solution was substituted for the gel. This was followed by 3 weeks of control with the gel again in the pouch.

RESULTS. No gastro-intestinal disturbances (vomiting, diarrhea) occurred in these studies. The data of individual days are too extensive to be shown, but they agree closely with the averages reported.

In the first series, during the feeding of 1.5 per cent $NaHCO_3$, small increases in volume (247 to 260 and 114 to 117 cc.) and in total chloride (47.8 to 52.1 and 25.7 to 28.0 m.eq.) were seen in both dogs during the first 9 hours after the test meal. The remaining 15 hours showed a decrease of volume in both dogs (285 to 214 and 56 to 51 cc.) with a decrease in total chloride (51.3 to 39.1 m.eq.) in one, while the other was unchanged (14.7 m.eq.). The average net result for 24 hours was a decrease in the volumes of secretion in both dogs (532 to 474 and 171 to 168 cc.), a decrease of total chloride (99 to 91 m.eq.) in one, and an increase (40 to 43 m.eq.) in the other. These variations are shown as percentages of change in figure 1, dogs 3 and 4.

Averages of the data of the second series (dogs 1 and 2, receiving 2 per cent $NaHCO_3$) are shown in table 1. These values are averages of 3 consecutive days, usually Wednesday, Thursday and Friday, and represent the results of that week. "Day" collection periods are the sum of the 3 separate collections made at 11 a.m. and at 2 and 5 p.m. The feeding of $NaHCO_3$ produced increases in volume, total chloride and free acid during the first 9 hours and were accompanied, with one exception, by slight decreases in total base. Despite a general decrease of all values in the following 15 hours, there was a net 24 hour increase in volume, total chloride and free acid, and a decrease in total base. The percentile differences in the values of the averaged 2 weeks of $NaHCO_3$ feeding from the averaged 2 weeks of control are shown in figure 1, dogs 1 and 2. The feeding of water instead of a solution of $NaHCO_3$ produced values only slightly higher, in a few instances, than controls. The distribution of the averaged secretory values of this series into individual collection periods is shown in figure 2. Increases in the values of the earlier periods (1 and 2) and decreases in the night period (4) are clearly evident. Table 2 shows no significant change in the hematology of these animals. The usual reciprocal relationship between plasma chloride and carbon dioxide capacity during $NaHCO_3$ feeding is seen in the small decrease (average 5.8 and 1.8 m.eq.) in the former and small increase (average 4.2 and 4.4 vol. per cent) in the latter.

The third series, in which 4 per cent $NaHCO_3$ was substituted for aluminum hydroxide gel in the pouch, showed changes similar to those seen when $NaHCO_3$ was fed. The average changes are shown as dogs 5 and 6 in figure 1. The pH of pouch contents at the time of collection varied between 5.3 and 7.8 (Beckman glass electrode) when $NaHCO_3$ was in the pouch and between 3.9 and 4.3 when aluminum hydroxide gel was present.

DISCUSSION. The objective of the technic used in these studies was to approach normal physiological conditions as closely as possible while allowing *continuous* observation of the secretory activity, rather than observation for a

few hours, following a test meal. In this direction are the following: *a.* The Cope pouch dog lives more normally than a pouch dog which requires bandages,

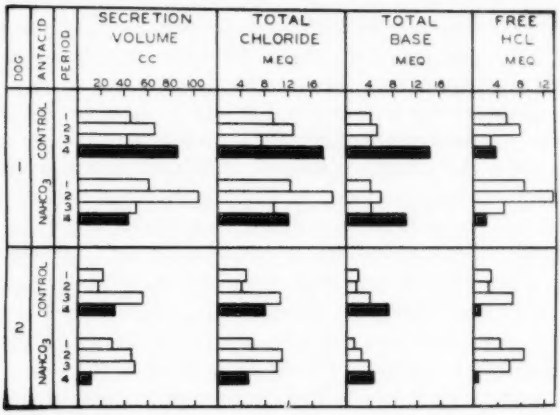

Fig. 2. The average secretory response of the Cope pouch during two weeks of feeding 50 cc. of 2 per cent $NaHCO_3$, t.i.d., compared with the average response during two weeks of control. The comparison is by collection periods 1 to 4, which are respectively, from 8–11, 11–2, 2–5 o'clock in daytime (open bars) and at night from 5 p.m. to 8 a.m. (black bars).

TABLE 2

Hematology and blood chemistry of the Cope pouch dog during the experiments shown in table 1

DATE	R.B.C.	W.B.C.	CELL VOL.	O_2 CAP.	Hb.	CO_2 CAP.	PLASMA CHLORIDE	DOG WT.
				Dog 1				
	$\times 10^6$	$\times 10^3$	*per cent*	*vol. per cent*	*gms.*	*vol. per cent*	*m.eq./l.*	*kgm.*
2/ 7/42	5.74	7.75	46.5	23.0	17.1	41.2	111.6	10.6
2/14/42	6.75	14.10	48.0	22.6	16.9	45.2	106.8	10.7
2/21/42	7.47	7.50	48.0	23.1	17.2	40.2	115.8	10.5
2/28/42	6.75	10.85	48.0	23.0	17.2	44.5	109.0	11.3
3/28/42	6.45	10.55	47.5	23.2	17.3	48.0	114.0	11.0
				Dog 2				
2/ 7/42	6.23	20.45	43.0	21.6	16.2	45.6	113.0	16.0
2/14/42	7.12	12.75	44.0	20.2	15.1	49.0	111.4	15.8
2/21/42	6.92	8.40	44.0	21.3	15.9	49.7	114.8	15.4
2/28/42	6.68	11.30	44.0	21.6	16.2	55.2	112.8	15.4
3/28/42	6.19	13.00	43.0	21.5	16.1	47.5	112.4	14.6

collection vessels, and restraints. *b.* A bulk of material is present in the pouch, as in the normal stomach during digestion, and its pH is not far removed from that of normal gastric contents. This avoids the abnormal condition of a relatively empty pouch in which the mucosa is in constant contact with a highly

corrosive, acid secretion. *c*. The test meal is not an interposed factor in the animal's normal routine, but is a part of that routine. *d*. Saline injections, known to influence secretion (13), and the inclusion of NaCl in the drinking water, to maintain acid-base balance, are avoided. *e*. The use of aluminum hydroxide gel within the pouch eliminates disturbances of the acid-base balance which may attend use of absorbable antacids (14).

The values obtained by this method of study may deviate slightly from absolute values: *a*. Aluminum hydroxide, a protein precipitant, removes most of the protein from the filtrate analyzed. Komarov and Komarov (15) have shown that pepsin is precipitated. *b*. The possibility of partial absorption of pouch contents is present even though the stomach is known to absorb most substances poorly. This possibility is common to all pouch studies. However, chloride appears to be unabsorbed by the gastric mucosa (16) and our studies support this observation. Significantly, we find that the amounts of the constituents secreted during a 9 hour control period do not vary when collections from the pouch are made hourly or at 3 hour intervals. Further, the values during control periods are reproducible from week to week with only slight variations. Those factors which may tend to introduce minor errors largely cancel out when the effects of a variable are determined by comparison of the differences between control and experimental values.

The effects of $NaHCO_3$ on the pouch secretion may be evaluated by considering figure 1. In all 6 cases the volumes during the first 9 hours showed increases ranging from 2.5 to 38 per cent. At the same time 5 dogs showed increases in total chloride which paralleled the volume changes, while dog 5 showed a slight decrease. To offset these general increases there were in every case, except the unchanged total chloride of dog 4, decreases at night which ranged in volume from 8.8 to 61 per cent, and in chloride from 20 to 37 per cent. The net results for 24 hours ranged from insignificant changes to marked increases in dogs 1 and 2. The daytime increases were characterized by the appearance of relatively larger amounts during the earlier periods of collection as seen in figure 2. This shift was typical of every case in the three series.

Further changes effected by $NaHCO_3$ are seen in the total base and free HCl variations of the second series. Figure 1, dogs 1 and 2, shows that there are significant net decreases of 12.5 and 38.0 per cent in total base, with marked net increases of 47 and 62.5 per cent in free acid. Thus in these experiments $NaHCO_3$ acts to elicit a higher percentage of the total chloride as free acid. This meets the need of more free acid to neutralize the $NaHCO_3$.

It becomes evident now that neither fractional gastric analyses nor pouch studies for a few hours following a test meal provide adequate data to reveal the effect of antacids on gastric secretion. Previous reports have shown that an increase in *acid concentration* may occur. Our data show at least two factors contributing to the "rebound" effect produced by $NaHCO_3$: *a*. The *amount* of acid secreted is increased. *b*. A shift occurs in secretory activity to greater amounts at earlier periods after the test meal. Both factors may operate in a given case, but our data show further, that even when *a* is relatively small, *b* may still be quite marked.

Considered as a whole these data suggest that the "rebound" acid concentration observed by fractional analysis after the ingestion of $NaHCO_3$ results primarily from the shift in gastric secretory activity. This brings a greater part of the secretion elicited by a test meal into the stomach at an earlier period and is accompanied by an increase in the amount of acid secreted. Since the stomach empties more rapidly under the influence of $NaHCO_3$, the factor of diminished dilution, as originally suggested, may then further increase the acid concentration.

The mechanism of the changes in gastric secretion observed in this study awaits clarification. Among the possibilities are the following: Sodium bicarbonate may cause secretory stimulation directly by local action on the mucosa, or indirectly by removal of acid inhibition (13) through neutralization. Or, perhaps, the small changes in total chloride and CO_2 capacity of the blood may be significant. Finally, the possibility of a humoral mechanism cannot be excluded. The similarity of the effects seen when the $NaHCO_3$ is fed and when it is placed in the pouch indicates that the mechanism involves more than a strictly local action on the parietal cells.

SUMMARY

Changes produced in gastric secretion by a 1.5 and a 2 per cent solution of $NaHCO_3$ administered three times daily in doses of 50 cc. by stomach tube, or a 4 per cent solution placed directly in the pouch were studied by use of a new technic employing the recently described Cope pouch dog.

Sodium bicarbonate produced an increase in the gastric secretory activity during hours immediately following a test meal, with a partial compensatory decrease during later hours. The role of these factors in "rebound" or "secondary" gastric acid secretion, as observed in fractional gastric analysis, is discussed.

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STIMULATION OF GASTRIC SECRETION BY NEURINE[1,2]

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Intramuscular injection of an extract of yeast stimulates gastric secretion in dogs according to Williams, Cox and Nash (1). Additional (unpublished) work has confirmed the original suggestion that the active agent is not histamine. Attempts to isolate this yeast principle have been unsuccessful.

Another approach to the problem of identifying the active yeast principle was by testing compounds known or suspected to be present in yeast. Of substances

TABLE 1

Effect of injection of 2.5 mgm. of neurine bromide (= 1.5 mgm. of neurine base) per kilo body weight on gastric secretion in dogs

PERIOD	VOLUME	FREE HCl	TOTAL ACID
	cc.	*meq.*	*meq.*
30 minutes preceding injection.....	8.2 ± 1.4	0.10 ± 0.04	0.24 ± 0.08
First 30 minutes after injection.....	42.2 ± 4.2	1.30 ± 0.19	2.00 ± 0.29
Second 30 minutes after injection.....	31.3 ± 3.5	0.93 ± 0.14	1.47 ± 0.23
Third 30 minutes after injection.....	19.0 ± 2.3	0.40 ± 0.08	0.57 ± 0.13

so far tested, including choline chloride, beta-alanine, glutathione, and neurine bromide (Hoffman-La Roche), only the last was definitely active. All substances tested were injected intramuscularly in a dose of 2.5 mgm. per kilo body weight.

Data of 17 experiments with neurine bromide on 4 different dogs with gastric fistulas are summarized in table 1, which also records the probable errors of the means.

We have shown previously (1) that there is no significant increase in volume, free hydrochloric acid, or total acid following control injections of 0.9 per cent sodium chloride in such dogs. However, after injecting neurine increases do occur in all three values. When the increase is compared with the error of the mean, using the table of t given by Fisher and Yates (2), the probability of the change being due to chance is less than 0.01 in every comparison during the first and second 30 minute periods following the injection.

The increase of total acid in the first 30 minute period after injection of neurine is comparable with that obtained following a dose of about 0.01 mgm. of

[1] This study was supported by a grant from Standard Brands, Inc.

[2] Data of this paper are taken from a thesis presented by Charles F. Hoffman in partial fulfillment of requirements for the degree of Master of Science, March, 1942.

histamine per kilo body weight, which has been found previously to be just above the threshold dose. In 6 experiments we have injected a smaller dose of neurine bromide (1.5 mgm. per kilo body weight) with results which are not statistically significant. The barely effective dose of neurine (which may be taken as 1.5 mgm. of neurine hydroxide per kilo body weight) is, therefore, about 150 times as large as that of histamine base.

It should be noted, however, that the effect of neurine is much more prolonged so that at the threshold dosage stated the total increase in volume and acid are more than double the increments after histamine. The prolonged action of neurine cannot be due to reabsorption from the gastric juice, since the latter was removed regularly by catheter.

The only report we have found of collection of gastric juice following injection of neurine is that of Miyazaki (3), who injected subcutaneously 25 mgm. or more of neurine per kilo body weight in order to demonstrate the subsequent presence of neurine in the gastric juice. He gave no data on the volume or acidity of secretion, however.

SUMMARY

Neurine, as bromide, when injected intramuscularly in dogs stimulates the secretion of fluid and acid by the stomach. The threshold dose of neurine base is about 150 times larger than that of histamine, but its effect is more prolonged.

In approximately the same dose of 2.5 mgm. per kilo body weight, choline chloride, beta-alanine and glutathione were ineffective.

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ANAEROBIC SURVIVAL OF ADULT ANIMALS[1, 2]

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The brain is regarded chiefly as an aerobic organ because anoxia causes rapid loss of cerebral functions. Cerebral energy may, however, be obtained anaerobically as the brain is able to split carbohydrate to lactic acid. Previous work on the brain of newborns of several mammalian species (1, 2) reveals that energy obtained by glycolysis is effective in supporting cerebral functions for a considerable period of anoxia. Injections of iodoacetate and fluoride (3) were found to shorten the survival period in nitrogen by preventing the breakdown of carbohydrate to lactic acid. It was concluded that though the rate of glycolysis in the newborn is slower than in the adult this slow rate appears adequate to support the lower metabolic demands of the infant brain for a considerable period of time in the absence of oxygen. It is the purpose of the present investigation to study this anaerobic production of cerebral energy in adult dogs and cats and to evaluate its significance for survival in these animals with the relatively high rate of cerebral metabolism characteristic of adults.

METHOD. Eight per cent oxygen in nitrogen was used in most of the experiments on dogs because the degree of anoxia produced by this gas mixture results in a survival period of at least one hour in these animals when anesthetized with pentobarbital, 25 mgm. per kilo. After trying various doses of iodoacetate from 25 mgm. to 49 mgm. per kilo, a dose of 32 mgm. was found to be most suitable because it permitted survival of sufficient duration when the anesthetized dogs were respiring air. In all instances the persistence of respiratory movements was employed as the criterion of survival. Three groups of dogs were used: the first group was subjected to the respiration of 8 per cent oxygen, the second to the injection of iodoacetate and the third to anoxia after the injection of iodoacetate.

Cats anesthetized with pentobarbital, 25 mgm. per kilo, injected intraperitoneally were used to determine the effect of 11 per cent and 8 per cent oxygen on brain lactic acid. Three groups of animals were studied: the first were controls, the other two respired either 11 per cent or 8 per cent oxygen for one hour. The brain was exposed through scalp and cranium. Then each animal received a slow intravenous injection of iodoacetate, 75 mgm. per kilo, in an attempt to inhibit glycolysis. Samples of brain and blood were taken and their lactic acid contents were determined by the method of Friedemann, Cotonio and Shaffer (4).

In another series of observations on dogs the effects either of the respiration of 8 per cent oxygen or the injection of 32 mgm. per kilo of iodoacetate on cerebral

[1] Aided by a grant from the Albion O. Bernstein Memorial Fund.

[2] Preliminary report appeared in Federation Proceedings **1**: 40, 1942.

arterio-venous oxygen differences were studied. The blood was analyzed for oxygen by the method of Van Slyke and Neill (5).

RESULTS. Table 1 summarizes the effects of the respiration of 8 per cent oxygen in nitrogen and of iodoacetate injection on the survival period. It is evident that the combined effects of anoxia and iodoacetate greatly shorten the survival period.

Table 2 discloses that with the respiration of 11 per cent oxygen the concentration of cerebral lactic acid rises above the control value. With 8 per cent

TABLE 1

Survival time of adult dogs with 8 per cent oxygen, iodoacetate and both

CONDITION	NUMBER OF DOGS	SURVIVAL TIME	
		Range	Average
		minutes	*minutes*
8% oxygen	5	60–271	151
Iodoacetate (25–49 mgm./kgm.)	17	49–240	127
8% oxygen + Iodoacetate (25–49 mgm./kgm.)	10	1½–10	4.3

TABLE 2

Lactic acid, milligrams per cent in brain and blood of cats

CONDITION	LACTIC ACID, MGM. PER CENT		
	Brain		Blood
Control	23		
11% oxygen	24	32	23
	35	43	19
	47	48	
8% oxygen	25	32	24
	44	44	30
	48	59	
	62	84	

oxygen brain lactic acid values are still higher. The table presents typical results of a long series of experiments. The value for the controls, 23 mgm. per cent, is the average obtained on 10 animals. The averages for 11 per cent and 8 per cent oxygen are 38 mgm. per cent and 50 mgm. per cent respectively. The values for blood lactic acid are not significantly raised by the respiration of these gas mixtures.

Table 3 presents the effects on 3 of the 6 dogs exposed to the respiration of 8 per cent oxygen. In every instance the cerebral arterio-venous oxygen difference decreases and does so progressively with time. Three observations of the 5 made after the injection of iodoacetate reveal that the initial cerebral arterio-venous oxygen difference is increased after injection.

Discussion. The results reveal that during partial anoxia the brain of the adult dog obtains sufficient energy from the anaerobic cleavage of carbohydrate to prolong significantly the survival period. When glycolysis is inhibited by the injection of iodoacetate in dogs respiring 8 per cent oxygen the survival period is shortened. Additional proof of the development of energy from carbohydrate during anoxia was obtained from a quantitative analysis of samples of brain and blood for lactic acid. In these animals the concentration of lactic acid in the brain was higher than in controls not subjected to anoxia. The cerebral lactic acid originated in the brain and did not diffuse to the brain from the blood because the concentration of lactic acid was higher in the cat brain than in the blood. The maintenance of the higher concentrations of lactic acid in the brain than in the blood may be accounted for by a certain degree of cerebral capillary impermeability to electronegative substances including lactic acid (6). The decrease of the cerebral arterio-venous oxygen differences in dogs respiring 8 per cent oxygen indicates that the respiration of this gas mixture does

TABLE 3

Arterio-venous oxygen difference after 8 per cent oxygen or iodoacetate

Volumes per cent

CONDITION	CONTROL	11 MINUTES	31 MINUTES	60 MINUTES
8% oxygen	11.3	7.3	5.8	
	6.9	4.3		2.6
	7.7		3.4	
Iodoacetate, 32 mgm./kgm.	6.8	8.7		
	6.8	9.3		
	7.0	10.2		

not furnish adequate supplies of oxygen to the brain. In every instance the cerebral arterio-venous oxygen difference diminishes during anoxia. The smaller arterio-venous oxygen differences are caused by a more rapid cerebral blood flow, a response to cerebral anoxia. In unpublished experiments on dogs anesthetized with Dial the respiration of 11 per cent oxygen had no effect on the cerebral arterio-venous oxygen differences. In cats anesthetized with chloralose, Courtice (7) observed that the cerebral arterio-venous oxygen difference did not begin to diminish until the respired oxygen fell to 15 per cent. The different sensitivities to anoxia exhibited with barbiturate and chloralose may be attributed to the fact that chloralose depresses the central nervous system and the respiratory centers less than do the barbiturates. Acceleration of circulation rate in response to cerebral anoxia may therefore develop with a lesser degree of oxygen deprivation under chloralose anesthesia.

The injection of iodoacetate increased the cerebral arterio-venous oxygen difference in dogs breathing room air. It is not likely that iodoacetate increased cerebral oxygen consumption but rather cerebral blood flow was slowed perhaps due to the effect of iodoacetate on the heart or capillaries. Irrespective of any

change in the rate of cerebral circulation, it should be noted that the oxygen intake continued despite a probable inhibition of cerebral glycolysis.

It is of some interest that after trial of various concentrations of iodoacetate a dose of 32 mgm. per kilo was chosen as the concentration compatible with adequate survival in air yet assuring rapid exitus while respiring 8 per cent oxygen. Henderson and Greenberg (8) have previously determined that iodoacetate in doses of 33 mgm. per kilo prevented the accumulation of lactic acid in the blood despite prolonged anoxia.

The brain probably employs more than one path for the oxidation of carbohydrate in the formation of energy. By one path, designated as Path I, glucose breaks down to pyruvic acid before oxidation to carbon dioxide and water, the Embden-Meyerhof scheme. But when this path is blocked by iodoacetate, the oxidation to carbohydrate continues by another path, Path II. There are, therefore, at least 2 paths for the oxidation of carbohydrate. On the other hand, during anaerobiosis, the brain can obtain energy only by the first path and the formation of lactic acid. The injection of iodoacetate during anaerobiosis therefore robs the brain of its only remaining source of energy; the formation of lactic acid by Path I is inhibited and cerebral function ceases. Both aerobic and anaerobic production of cerebral energy is greater in the adult than in the infant brain (1, 2, 9). The increase in the aerobic processes, however, is larger than in the anaerobic. As a result of this shift to aerobic processes the adult is less tolerant to anoxia than is the infant.

SUMMARY

A study was made in order to evaluate the significance of the anaerobic development of energy from the cleavage of carbohydrate for the maintenance of cerebral function. 1. In dogs anesthetized with pentobarbital and respiring 8 per cent oxygen in nitrogen the cerebral arterio-venous oxygen differences decrease indicating an inadequate supply of oxygen to the brain. 2. Lactic acid accumulates in the brain of cats anesthetized with pentobarbital and subjected to the respiration of 8 per cent oxygen, as the cleavage of carbohydrate affords energy for the maintenance of cerebral functions during anoxia. 3. The injection of iodoacetate, 32 mgm. per kilo, which inhibits the cleavage of carbohydrate shortens the survival periods of dogs anesthetized with pentobarbital and respiring 8 per cent oxygen. It may, therefore, be concluded that during a period of partial anaerobiosis the brain of the adult dog or cat obtains some of the energy required for its maintenance by the anaerobic splitting of carbohydrate and the formation of lactic acid. It should be pointed out however that the ability of the adult to withstand anoxia is much more limited than the infant.

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ESERINE, ACETYLCHOLINE, ATROPINE AND NERVOUS INTEGRATION

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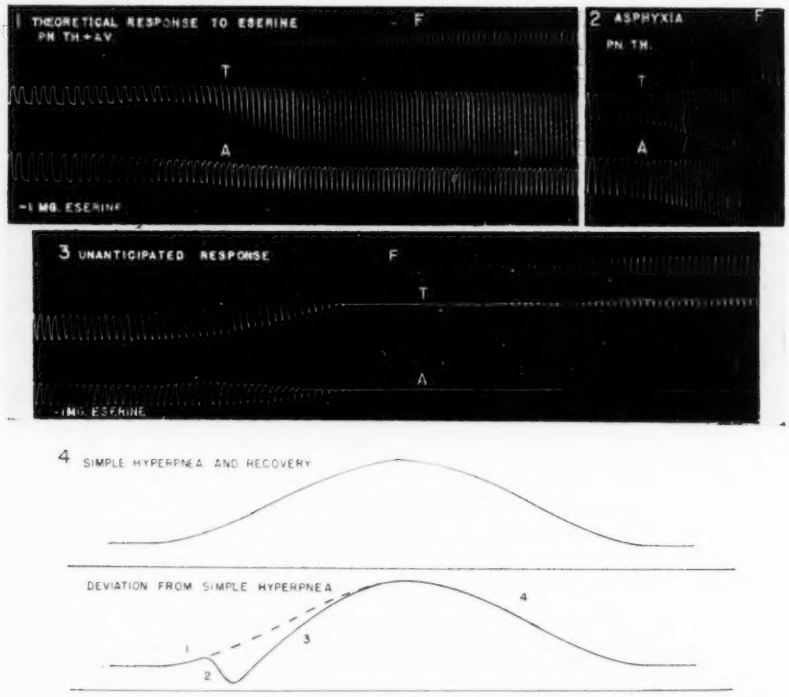
According to the "humoro-electrical" theory, activation of nerve cells transpires in two stages—humoral and electrical (Gesell, Brassfield and Hansen, 1942). Impulses impinging on the nerve cell release the neurohumor. Acetylcholine by virtue of its rate of destruction pools at the dendrites and cell body and provides an adjustable electrochemical voltage, the intensity of which is determined by the size of the pool. A resulting continuous current leaves the neuron at the axon hillock and fires the neuraxon at a frequency proportional to its intensity. Artificially administered extrinsic acetylcholine on reaching nerve cells, adds to the existing intrinsic acetylcholine and potentiates the reflexes motivated by the existing physiological bombardment. Eserine protects the intrinsic acetylcholine liberated by physiological bombardment and produces similar potentiation of physiologically motivated reflexes (Gesell, Hansen and Worzniak, 1943). Atropine by counteracting the effects of acetylcholine exercises a depotentiating influence. Because the effects of acetylcholine, eserine and atropine are necessarily linked with prevailing synaptic bombardment, they offer unusual promise as chemical tools in the unravelling of the mechanisms of nervous integrations.

PROCEDURES. Our methods are described in part in earlier studies on humoral intermediation of nerve cell activation (Gesell, Hansen and Worzniak, 1943). In the present observations (all on the dog) the abdominal and costal respiratory movements and the contractions of the accessory respiratory muscles of the face were recorded. This was essential to show qualitative changes in breathing which ordinarily fail to reveal themselves in the spirometer record alone. The thoracic and abdominal movements (change of girth) were recorded with a modification of the torsal band method (Gesell and Moyer, 1935). The facial muscular contractions were registered with a vertical writing point attached to the fold of skin at the angle of the mouth. Downstroke represents inspiration in the band records and expiration in the facial tracings unless otherwise specified. Facial, thoracic, abdominal and spirometer records are labelled F, T, A and S.

RESULTS. 1. *Respiratory response to eserine conforming with theoretical expectations.* Granting that breathing is synaptically motivated, eserine, by virtue of its anticholinesterase activity might be expected to produce the same effects as acid, namely, a co-ordinated hyperpnea. But eserine produces two types of response illustrated in figure 4—a simple hyperpnea such as may be expected from excess of CO_2 and an unanticipated deviation which differs in striking and characteristic ways to be described below. Of the two changes the expected

result seen in figure 1 is by far the less common. This observation was made during pneumothorax plus constant ventilation to assure a uniform pH. It compares in essentials with the response to simple asphyxia during pneumothorax minus ventilation to assure a diminishing pH illustrated in figure 2. The records from above down are facial, chest and abdominal movements. The slowness of the development of hyperpnea during eserine poisoning and the protracted recovery (not shown) seem merely to reflect a very gradual accumu-



Figs. 1–4

lation and disappearance of acetylcholine. Return to normal breathing may require 1 to 3 hours.

2. *Quantitative deviations from the theoretical response to eserine.* Deviation from the expected response to eserine (see schema, fig. 4) consists of an early interruption of the initial hyperpnea which divides the complete response into four instead of two phases: 1, a relatively brief hyperpnea; 2, a subsequent diminishing ventilation; 3, a relatively prolonged period of increasing ventilation, and 4, a protracted return to normal. See spirometer tracing of figure 6 for stages 1, 2 and 3. Stage 4 is omitted.

Abolition of pulmonary ventilation in stage 2 (see figs. 10 and 11) would indicate a complete paralysis of the respiratory center. The sudden appearance

of powerful sporadic inspirations scattered among the periods of apnea or during shallow breathing in that same stage in figures 5, 6 and 7 however speak for a highly excitatory condition of the respiratory center. In a like manner the diminution of tidal air in figure 11 agrees with a depression of the center, but the concurrent accelerated frequency suggests the reverse. The final apnea, therefore, cannot be interpreted as an unquestionable paralysis. The significance of the changes in pulmonary ventilation is more effectively approached by a consideration of the qualitative changes in breathing.

3. *Qualitative changes in breathing produced by eserine.* The qualitative changes which eserine produced upon the respiratory act proved far more significant than the quantitative fluctuations in pulmonary ventilation because they revealed highly significant differential effects of nervous imbalance. At the height of eserine poisoning breathing is radically changed. The accessory respiratory muscles of the face and neck contract vigorously, as if the animal were suffering from intense asphyxia, while at the same time the torso shows no respiratory movements whatever. The condition develops slowly. As one muscular activity diminishes another increases with the resultant effect that respiration changes from a normal and predominantly inspiratory type to a predominantly expiratory type. Eventually torsal respiratory movements disappear while facial inspiratory contractions persist at maximum presenting a different type of inspiratory breathing from that of eupnea. With recovery these events are reversed. Facial, chest and abdominal movements as well as pulmonary ventilation were therefore recorded. Figures 5, 6, 7, 14, 15, and others illustrate some of the outstanding points in this extraordinary phenomenon.

Figure 6 is our most complete illustration, lacking only the last stages of an uneventful recovery back to eupnea. Two milligrams of eserine were injected intravenously at *A'*. Following the initial hyperpnea at *B* thoracic and abdominal movements and pulmonary ventilation diminish after which breathing becomes decidedly irregular in both costal and abdominal segments. Irregularity of breathing is ushered in by a progressive constriction of the chest. At maximum constriction normal respiratory rhythm in the abdominal segment is abolished. Only occasional sporadic inspirations break through. In the chest however respiratory contractions continue without interruption and increase in amplitude at an early stage of poisoning. Normal respiratory rhythm in the abdominal segment returns only after the chest has begun to relax. Abdominal movements then increase in strength along with the chest. Irregularity of breathing disappears as the expiratory activity of the chest weakens.

Dominance of costal breathing results from at least two changes in muscular activity: 1, a weakening of diaphragmatic contractions, and 2, a strengthening of costal expiratory contractions. Of the acquired qualitative changes in breathing, costal expiratory activity appears first and facial inspiratory contractions appear second, after an interval of about 3.5 minutes. Both activities increase in intensity for another 4 minutes up to point *C*. This marks the peak of eserine poisoning beyond which facial inspiratory and costal expiratory activity diminish. Facial contractions disappear within 9 minutes, at point *D*.

Costal expiratory activity is still present at the end of the record. The order of appearance of costal expiratory and facial inspiratory activity and the reversed order of diminution and disappearance of these activities is rather characteristic.

Several features of this response seem exceptionally pertinent. *Inspiratory* contractions of the diaphragm *weaken* as the *expiratory* contractions of the chest *increase*. *Inspiratory* contractions of the diaphragm *weaken* as *inspiratory* contractions of the face *increase*. Neither combination has the earmarks of co-ordination toward attainment of hyperpnea. On the other hand the simultaneous increase of inspiratory and expiratory activity in the chest is indicative of a localized co-ordination. Failure of the diaphragm seems to be the major disco-ordination.

Figure 14 shows an exceptionally orderly series of events in the three major alterations in a relatively slowly breathing animal. Irregularity of breathing is missing despite intact vagal reflex paths. Diaphragmatic contractions diminish and facial contractions increase. After an initial decrease the thoracic contractions increase along with the facial contractions. The increase in thoracic activity is primarily expiratory. As noted in figure 6, there are opposing and co-ordinated changes in activity in three groups of muscles. There is an increasing expiratory activity in the chest, and an increasing inspiratory activity in the face associated with a decreasing inspiratory activity in the diaphragm. Deficient diaphragmatic movement is compensated in part by increasing costal movement, mainly expiratory. Tidal air increases after an initial depression despite abdominal inactivity. Breathing changes from a predominantly inspiratory type toward an increasingly expiratory type. Ten milligrams of atropine injected into the vertebral artery brings on a sudden reversal of effects differing from ordinary recovery mainly in the abruptness of the changes. Facial contractions cease, active expiratory contractions disappear (note the sudden drop in the costal expiratory level), diaphragmatic contractions suddenly return and the animal is back to the inspiratory type of breathing.

In general the spirometer records tell little about the qualitative changes in breathing but on rare occasions they differentiate in a single tracing the active and passive phases of inspiration and of expiration. Such was the fortunate case in figure 15. The dog was vagotomized and breathing was relatively slow. During eupnea inspiration is certainly active and expiration most probably passive—at least predominantly passive. This dominance of inspiratory activity persists throughout the initial hyperpnea, *A*, and the following hypopnea, *B*, up to point *C*. After point *C* the respiratory cycle divides itself into five distinct phases: 1, active inspiration; 2, passive expiration; 3, inactive expiratory pause; 4, active expiration; 5, passive inspiration. The quiescence of the torso during the expiratory pause produces a series of notches and a resulting demarcation line which reveals the relative intensities of contraction of the inspiratory and expiratory muscles. After the sudden reduction of respiration up to point *C* ventilation increases up to point *E* where the effects of eserine are interrupted by an intravenous injection of atropine. From *C* to *D* ventilation increases

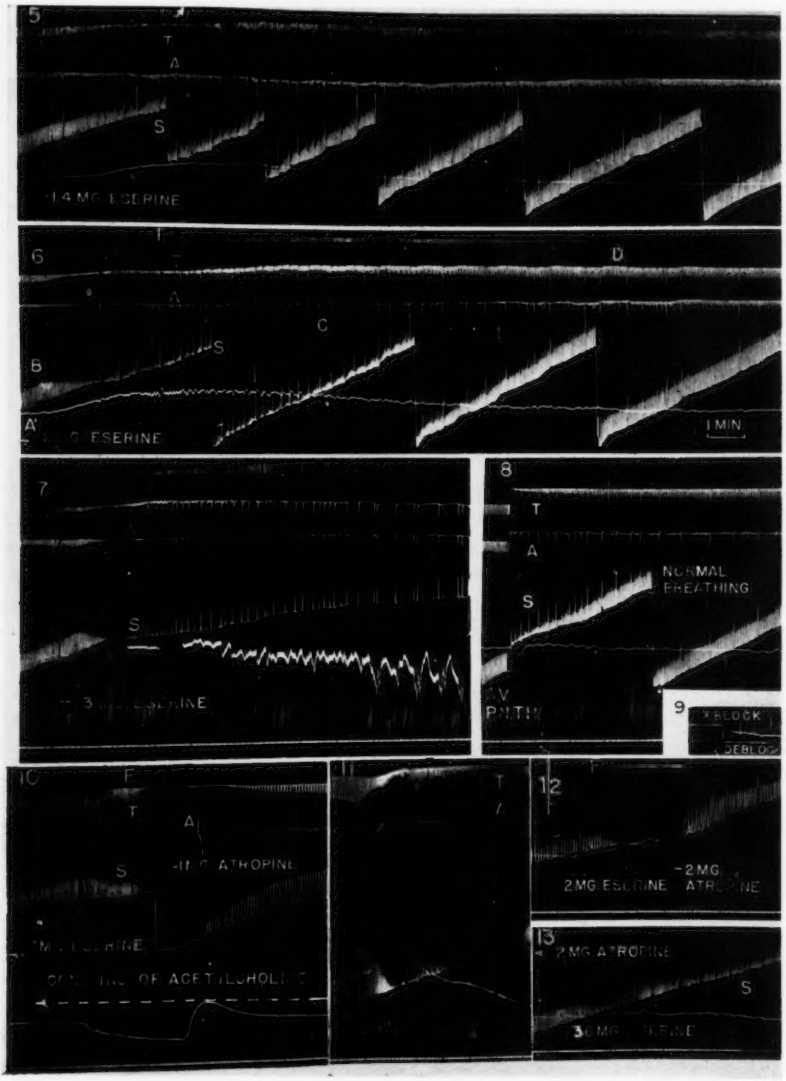
despite a steadily weakening inspiration. The adjustment is made by a steadily increasing expiration. From *D* to *E* breathing continues to increase despite a slowly diminishing expiratory activity. The adjustment now comes through an increasing strength of inspiratory contraction. A sudden and striking transformation of breathing occurs at *E* comparable to a rapid and condensed recovery. Thus frequency of breathing diminishes. The expiratory component disappears. Accessory inspirations vanish. Diaphragmatic contractions return in force. Breathing is back to the predominantly inspiratory type.

What do these changes signify, individually and collectively? The increasing and decreasing force of facial contractions are a perfect duplication of hypercapnic hyperpnea and recovery. They agree with a progressively increasing and decreasing pooling of acetylcholine in the facial accessory arcs. Increasing and decreasing intensity of thoracic expiratory contractions agree with an increasing and decreasing pooling at the corresponding expiratory neurones. It would be stretching the point to attribute the irregularity of torsal inspiration noted in figure 6 to a coinciding irregularity of pooling in the torsal inspiratory arcs. There is no evidence that eserine, by itself, is capable of such effects. It would seem more logical to attribute the irregularity of breathing to sporadic changes of imbalance between the opposing half-centers and to accept the stronger contractions as the more reliable measure of the potential activity of the torsal inspiratory neurones. Working on that assumption we have noticed that the inspiratory contractions tend to divide into two groups, weaker contractions which decrease in intensity up to the peak of eserine poisoning and greater sporadic contractions which tend to increase in power as eserine poisoning increases (see figs. 5, 6 and 13). How are these diametrically opposite changes to be reconciled? Excessive expiratory activity could impress excessive inhibition on the inspiratory neurones via reciprocating connections. On the other hand an increased pooling of acetylcholine at the inspiratory neurone could account for the increasing intensity of those inspirations which sporadically break out of the imbalance (see Gesell and Hamilton, 1941, and review by Gesell, 1940). Were it not for the disco-ordinate potentiation of the expiratory neurones eserine hyperpnea might be as highly co-ordinated as hypercapnic hyperpnea.

While the increase of the larger inspiration does not always follow a smooth curve, such as illustrated in figure 13 the general tendency in that direction is unmistakable. On the whole smaller injections of eserine are more likely to produce irregularity of breathing and an increase and decrease of depth of the two groups of inspirations. Compare figures 5, 6 and 7 in which 1.4, 2.0 and 3 mgm. of eserine were injected. Large injections tend to prevent the appearance of the powerful inspirations as in figure 12 or to delay their onset as in figure 7.

4. *A possible rôle of bronchiole constriction in the respiratory response to eserine.* It has been suggested to us that a constriction of the bronchioles resulting from accumulation of acetylcholine at the pulmonary motor endings might impede the movement of air in and out of the lungs, at the same time allowing unimpeded contractions of the facial muscles which are not exposed to this mechan-

ical resistance. Two observations question the importance of bronchiole constriction—absence of powerful torsal respiratory efforts in severe eserine poisoning



Figs. 5–13

such as are ordinarily seen in all forms of mechanical asphyxia (pneumothorax, fig. 2, or clamping of the trachea) and the actual occurrence of deep breaths interspersed among the subnormal inspirations and apneas (figs. 5, 6 and 7).

Nevertheless it seemed desirable to seek more quantitative evidence by recording the effects of eserine during constant artificial ventilation administered during double open pneumothorax. Constant pulmonary ventilation has the purpose of eliminating acidity as a modifying factor and double pneumothorax assures freedom of movement to all groups of respiratory muscles. Should bronchiole constriction occur it can effect neither the ventilation of the blood, nor the extent of the contraction of the respiratory muscles. The customary hyperpneic activity of the facial accessory muscles and the simultaneous torsal apnea (fig. 3) show that the peculiar respiratory response to eserine must be sought in some detail other than bronchial constriction.

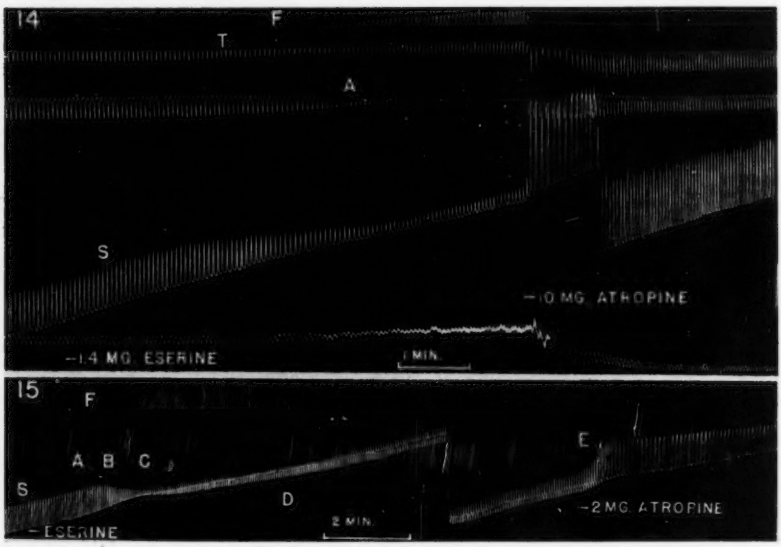
5. *Effects of size of injection of eserine.* The amount of eserine injected influences the respiratory response in several ways (see figs. 5, 6 and 7). One and four-tenths milligrams of eserine evoked no facial accessory response, 2 mgm. evoked a medium response and 3 mgm. a powerful response. The response appeared within approximately 5 minutes after the injection of 2 mgm. and within approximately 1.5 minutes after 3 mgm. Irregularity of torsal breathing and regularity of facial breathing occurred with all doses. The appearance of the sporadic deep breaths as mentioned above was momentarily delayed by the large injection. Normal respiratory rhythm persisted in both costal and abdominal segments after the smallest injection. With the intermediate dose of 2 mgm. the chest followed facial contraction consistently while the abdomen followed only sporadically. With the large injection the normal rhythm disappeared in both torsal segments, presenting an interesting picture of two simultaneous respiratory rhythms, a rapid normal rhythm in the upper facial arcs and a slow or partially blocked rhythm in the lower segments.

The earlier appearance of accessory contractions with large injections of eserine indicates a more rapid accumulation of acetylcholine at the facial neurones under the increased protection offered by those doses. Presence of torsal response and absence of facial response to the smallest injection (1.4 mgm.) indicates lighter bombardment of the facial arcs, i.e., a smaller release of acetylcholine requiring greater potentiation to bring it to threshold stimulation. More extensive elimination of torsal inspiratory movements with increasing doses of eserine indicates an increasingly disproportionate potentiation of the expiratory neurones and a correspondingly greater domination of the inspiratory neurones (see below).

6. *The factor of asymmetrical bombardment and asymmetrical potentiation of opposing half-centers.* Pronounced asymmetrical activity of the opposing half-centers during ordinary eupnea suggests a correspondingly pronounced asymmetry of bombardment. The successive order of appearance of inspiratory, expiratory and accessory contractions during asphyxia following upon artificially induced hypocapnic apnea would seem to reflect the relative intensities of the corresponding bombardments. Increasing anticholinesterase activity (hypercapnia in this instance) superimposed upon apnea must theoretically potentiate all existing bombardments and call additional respiratory muscles into play in the order named. Should the acetylcholine machinery of integration be sus-

ceptible to disco-ordinate (i.e., disproportionate) potentiation, let us say of the more lightly bombarded expiratory neurones, the inspiratory neurones must give way in part to the dominance of the superactivated expiratory neurones via reciprocal inhibition.

The existence of such a mechanism is actually suggested in the response of muscle to indirect stimulation of low and high frequency (Rosenblueth and Morrison, 1937). Eserine injected during the course of such stimulation was found to potentiate the effects of infrequent stimulation (explained by moderate accumulation of acetylcholine at motor end plates) and to paralyze the muscle to frequent stimulation (explained by excessive accumulation of acetylcholine). Preliminary experiments on the diaphragm agree with those of Rosenblueth and



Figs. 14–15

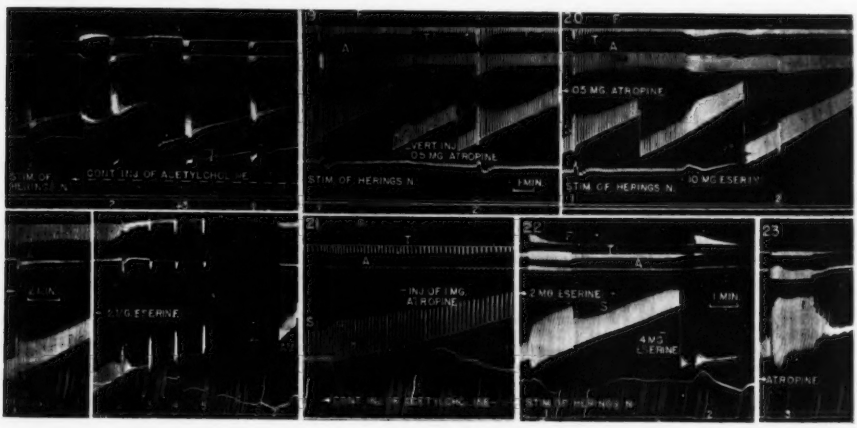
Morrison. We have reversed the procedure and sampled the response of muscle to electrical stimulation during the course of eserine poisoning. To mention a single example—the diaphragm was found to respond with a single twitch to a continuing stimulation of the phrenic nerve at a frequency of 45 per second and with a series of twitches to a stimulation frequency of 15 per second. Small and large concentrations of acetylcholine exercise comparable potentiating and paralyzing effects upon sympathetic and parasympathetic ganglia.

Since our findings on respiration could be explained by peripheral muscular as well as by central nervous action it was desirable to inquire into the problem of relative susceptibility of muscle and center and thus establish the main site of action of eserine.

7. *The site of disco-ordination of the respiratory act in eserine poisoning.*

Eserine unquestionably produces a central disco-ordination because the intensity of the electrical potentials of the phrenic nerve and the intensity of diaphragmatic contraction run parallel courses. When contractions cease electrical activity is missing and when contractions return and increase in volume the same changes in phrenic electrical activity occurs. The fact that the diaphragm will respond to artificial stimulation of the phrenic nerve at a time when physiological contractions and phrenic potentials are missing indicates that central integration is more sensitive to eserine than is simple intermediation at the motor end plate of the diaphragm. With larger injections however both muscle and center may be involved.

Parallel changes of expiratory nerve potentials and expiratory muscle activity give double assurance that the centers are importantly involved in the respiratory response to eserine poisoning.



Figs. 16–23

Absence of phrenic potentials during eserine hypopnea however must not be interpreted as *paralysis* of the inspiratory half-center unless reciprocal inhibition is eliminated. Evidence on that issue must be sought in other ways.

8. *Central paralysis of the inspiratory neurones versus reciprocal inhibition via excitation of expiratory neurones.* Inhibition and paralysis differ in that one implies a functional state and the other a loss of function. Reflex stimulation of the respiratory center should allow experimental differentiation. A paralyzed inspiratory half-center should be incapable of responding to an inspiratory stimulation (Hering's nerve) whereas an inhibited center should yield (see figs. 17 and 18). Although eserine had virtually arrested breathing in figure 18 stimulation of Hering's nerve produced a more powerful response than before. Breathing bursts out of apnea with a surprising suddenness and frequency and ceases without a sign of after-discharge. Only two breaths occurred during stimulation before eserine was injected as compared with 6 breaths during eserine poisoning.

The suddenness with which breathing stops at the end of stimulation is a most interesting phenomenon because eserine is generally regarded as favoring after-discharge. Fulton (1938) believes that after-discharge is highly susceptible to inhibition. Can it be that the excessive expiratory activity, as displayed in the chest, is reciprocally opposing the tendency of the inspiratory half-center to after-discharge? If so the "apnea" which obtains between stimulations is an indirect expression of excitation rather than a direct expression of paralysis, i.e., an indirect inhibition resulting from excessive and imbalanced stimulation of the expiratory neurones.

The extremely rapid breathing which stimulation of Hering's nerve produced may also be related to the dominant expiratory potentiation by eserine poisoning because stimulation of the predominantly expiratory excitatory vagus is known to intensify and prolong the expiratory phase of breathing thereby retarding rhythm; and stimulation of the predominantly inspiratory excitatory Hering's nerve (vagi cut) is known to intensify and prolong the inspiratory phase of breathing and thus retard rhythm. Simultaneous stimulation however does not produce an additive slowing. Instead, both phases of breathing are shortened and frequency increased. It is believed that stimulation of Hering's nerve in figure 18 adds a powerful inspiratory drive to a powerful expiratory drive already prevailing during eserine poisoning. In so doing it corrects a respiratory imbalance and forces the center to discharge at an accelerated rhythm.

Although a predominant potentiation of the expiratory neurones is sufficient reason for apnea, excessive eserine poisoning is capable of producing direct paralysis of breathing as well. This is seen in figure 18 where the potentiation, evident in the first four stimulations, suddenly gives way to paralysis as the intensity of poisoning advances (stimulations 5 and 6). Note the fall in blood pressure.

9. *Proprioceptive reflexes in eserine poisoning.* The central effects of acetylcholine and eserine are importantly modified by prevailing physiological reflexogenic support (Gesell, Hansen and Worzniak, 1943). Vagal proprioceptive reflexes are especially effective in molding the patterns of breathing during eserine poisoning. This is evident in the irregularity of breathing (figs. 5, 6 and 7), and the readiness by which irregularity is abolished by vagotomy or vagal block (fig. 9). Subsequent X deblocking re-establishes the irregularity. This influence of the vagus nerves seems to originate in the stretch reflex rather than the collapse reflex for if the lungs are maintained at subnormal volumes (artificial ventilation during pneumothorax, vagi intact) breathing continues with perfect regularity (fig. 8). If the lungs are now brought back to normal volume (discontinuance of artificial ventilation and pneumothorax) irregularity of breathing returns immediately.

Sustained pulmonary inflation to supernormal volume and simple eserine poisoning show analogous effects pertinent to our problem (Gesell, 1940). Both establish a dominating expiratory activity. Both hold inspiration in abeyance and create apneic pauses of varying duration. These pauses are interrupted by irregular breaths of varying depths, normal, subnormal, and supernormal.

Each breath transpires with accelerated velocity, expiration often retracing the inspiratory stroke. Presumably these analogous effects have similar integrations.

The dual excitatory mechanisms of vagal function hold that a stretched condition of lungs reflexly releases an increased amount of acetylcholine at both half-centers. Because more acetylcholine is released at the expiratory half-center, the inspiratory half-center is held in reciprocal abeyance. When, however, the inspiratory half-center breaks out of the dominance of the expiratory half-center it discharges violently because the superactivity of the inspiratory excitatory fibers has accumulated excessive amounts of acetylcholine about the inspiratory neurones.

Theoretically analogous conditions prevail in simple eserine poisoning. Acetylcholine accumulates in the inspiratory and expiratory neurones thereby increasing the potential power of every impulse impinging on both groups of cells. Disproportionate intensification of expiratory activity holds the inspiratory neurones in abeyance. When however they do discharge they do so under the driving force of excessive acetylcholine. Inspiration is therefore rapid and powerful. This produces a rapid inflation of the lungs and powerful reflexogenic release of acetylcholine at the inspiratory neurones which in turn is spared by the anticholinesterase activity of eserine. Inspiration gets out of control and reaches supernormal depths. The simultaneously intensified expiratory stretch reflex and the greater accumulation of acetylcholine at expiratory neurones intensifies the expiration which follows.

10. *A comparison of the effects of eserine and of acetylcholine.* Granted that eserine produces a gradual accumulation of acetylcholine at nerve cells, a slow and continuous injection of a weak solution of acetylcholine might be expected to produce approximately similar conditions. Acetylcholine was injected with a specially devised pump into the carotid artery (2 cc. per minute of 1/10000 solution) after having denervated the carotid bodies (fig. 16). The period of injection is indicated. The effects are remarkably similar to those of eserine in figure 18. There is a temporary increase of tidal air, a marked increase of frequency of breathing with a decided falling off of tidal air. The chest shows marked expiratory constriction, the abdomen passive dilatation and the chest movements are better conserved than the abdominal. There is the same augmented increase of respiratory response to faradic stimulation of Hering's nerve. Reflexogenic ventilation is trebled.[1]

11. *Antagonism between atropine, and acetylcholine or eserine.* The effects of atropine upon breathing during eserine poisoning are exceptionally striking. Respiration which has been completely stopped by excessive eserine poisoning is restored within a few seconds by small amounts of atropine (approximately 1 mgm.). When administered during milder poisoning atropine reverses each qualitative change in breathing which eserine had set up (see figs. 14 and 15).

[1] Schweitzer and Wright state that "The excitatory action of eserine cannot be due to its anticholinesterase action as it is the converse of the action of acetylcholine" (J. P. **89:** 196, 1937).

The highly active accessory respiratory muscles of the face stop contracting, the active expiratory contractions of the chest disappear, and the inactive or weakened diaphragm contracts with greater vigor. Similar antagonism exists between atropine and acetylcholine. When acetylcholine is injected continuously in dilute solution to simulate the conditions of eserine poisoning atropine restores torsal pulmonary ventilation if breathing has been suppressed (fig. 10). On the other hand if breathing has been increased, atropine diminishes pulmonary ventilation (fig. 21), agreeing with the findings of Miller, Stavraky and Woonton (1940) on the cerebral cortex.

This opposing action of atropine upon extrinsic acetylcholine is in agreement with the well recognized antagonism of the muscarine effects of acetylcholine and those of atropine in the parasympathetic system. Since it is highly probable that eserine increases the amount of extra-neurocellular acetylcholine the action of atropine during eserine poisoning may be analogous to that during extrinsic acetylcholine poisoning.

Theoretically atropine could produce significant effects at several points, at the myoneural junction, at synaptic junctions in the chemoceptor organs (v. Euler, Liljestrand and Zotterman, 1941) and in the centers. The elimination of the myoneural junction as a factor in the production of the typical respiratory response to moderate amounts of eserine eliminates this site of action of atropine in the reversal of eserine effects. This conclusion is also supported by our direct observations on nerve and muscle action potentials. When atropine is injected directly into the vertebral artery during eserine poisoning (fig. 14) the effects are more profound, and more sudden than when injected intravenously (fig. 15). The restoration of breathing within a period of two to three seconds indicates a central action of the drug. Comparable effects of atropine during eserine poisoning after denervation of the carotid bodies and double vagotomy supports this conclusion (not illustrated).

We are forced to believe, contrary to generally accepted opinion, that atropine is capable of exercising a powerful anti-acetylcholine and anti-eserine action in the nerve cells of the central nervous system. Our findings and those of Miller, Stavraky and Woonton (1940) corroborate those of Marrazzi (1939) in peripheral ganglia and make the observation general for all nerve cells.

12. *Central effects of atropine upon eupneic breathing and on the reflex response to faradic stimulation of Hering's nerve.* Small injections of atropine into the vertebral artery after denervation of the carotid bodies and double vagotomy diminishes breathing appreciably. In figure 19 eupnea was reduced approximately 20 per cent by 0.5 mgm. and artificial reflexogenic hyperpnea, produced by faradic stimulation of Hering's nerve, was diminished a comparable amount. Compare the pre- with the post-atropine response. Proper preconditioning with eserine will reverse the effects of atropine upon reflexogenic response (figs. 22 and 23). The first stimulation of Hering's nerve, administered about 15 minutes after an intravenous injection of 2 mgm. of eserine, showed a marked potentiation of the facial, costal and abdominal movements (control omitted). Four additional milligrams of eserine after stimulation no. 1 abolished chest and abdominal movements and introduced a series of minute facial contractions. Stimulation

no. 2 applied during this increased anticholinesterase influence produced a relatively smaller response of the chest and abdomen and a relatively larger response of the facial accessory movements than stimulation no. 1. This response is reversed by atropine in stimulation no. 3. Torsal movements are now increased more than they were in stimulation no. 2 and facial response is relatively diminished. According to our reasoning acetylcholine exceeded the limits of potentiation at the heavily bombarded torsal arcs in stimulation no. 2 but remained within the limits of potentiation at the more lightly bombarded facial arcs. Elevation of the threshold of stimulation by atropine abolished the paralytic action of high concentration of acetylcholine at the heavily bombarded arcs and kept the effects of the acetylcholine within the limits of potentiation. But at the facial arcs where acetylcholine had failed to reach paralytic concentrations in stimulation no. 2, atropine raised the threshold excessively and thereby weakened the stimulating effects of acetylcholine released during stimulation no. 3.

The simultaneous occurrence of both excitatory and depressing effects at individual functional units of the respiratory center from a single injection of atropine is interesting in view of the fact that central effects of both types are ascribed to atropine (Goodman and Gilman, 1941).

13. *The effects of preconditioning with atropine upon the respiratory response to eserine.* Atropine in quantities as minute as 1 mgm. raises the threshold of respiratory response to eserine enormously. Often the effects are mainly quantitative, at other times marked qualitative effects occur as well. Instead of the typical four phasic response eserine may produce a simple hyperpnea comparable to that of hypercapnia (fig. 20). A more perfect balance between half-centers is in some way attained. If this better balance is attributable solely to an increase of threshold it illustrates another simple means by which nervous integration can be strikingly modified.

Comments. The great value of eserine as a physiological tool lies in its accepted mode of action. Though non-stimulating in itself its end result is stimulation. Like the hydraulic dam which pools the energy of a flowing stream of water and thereby motivates hydraulic machinery, eserine pools the nervous power of a continuing stream of nerve impulses. Without primary disturbance in the volume of physiological bombardment it raises the level of acetylcholine and supermotivates the nervous machinery to which this humoral energy is hitched. Faced with this peculiar action it seems increasingly difficult to deny the steady or "tonic" nature of nervous forces (Gesell, 1940) or to defend a purely electrical and temporally rigid concept of synaptic "transmission".

It stands to reason that a powerful neuro-humoral drive calls for a proportionately powerful inhibition if one half-center is to give way to the other in rhythmical alternate activity. A unihumoral mechanism of nervous integration might conceivably meet such requirements on the assumption that acetylcholine is capable of producing either stimulation or inhibition depending upon where it is released—excitation if released at the dendrites and inhibition if released at some strategical counterelectromotive position such as the axon hillock or base of dendrite. Without disrupting the machinery of integration a

simultaneously increased anticholinesterase action at both excitatory and inhibitory dendrites might produce a co-ordinated hyperactivity such as is seen in hypercapnic hyperpnea. A dual humoral system of nervous integration based on the experiments of Marrazzi (1939) on autonomic ganglia is an alternate possibility.

SUMMARY

Among the outstanding observations on eserine were 1, a reinforcement of thoracic expiratory contractions; 2, a temporary irregularity of strength of torsal inspiratory contractions associated with subnormal pulmonary ventilation; 3, an initiation and strengthening of the facial accessory respiratory contractions.

These effects occur with vertebral injections after chemoceptor denervation and are therefore central.

Irregularity of breathing consisted of shallow breaths tending to weaken and of sporadically deep breaths tending to strengthen as eserine poisoning increased.

Large injections intensified all effects of eserine including the curtailment of the more shallow breaths. Complete curtailment left the powerful inspirations separated by apneas of variable durations.

Corresponding apneas were absent in the facial rhythm. Respiratory block is indicated.

Increasing intensity of costal expiratory, of torsal inspiratory and of facial accessory inspiratory contractions are thought to indicate a progressive pooling of acetylcholine at all nerve stations where bombardment and release of acetylcholine occurs.

Failure of the smaller inspirations to attain maximum intensity is attributed to a disproportionate potentiation of expiratory neurones structurally coupled with their opposing inspiratory neurones. Only those inspirations breaking free of the dominating reciprocal inhibition are thought to gauge the potential activity of the inspiratory cells.

The effects of eserine were duplicated with slowly injected extrinsic acetylcholine.

Atropine produced profound central effects: 1, reversal of the response to eserine and extrinsic acetylcholine, and 2, diminution of breathing motivated by intrinsic acetylcholine (fig. 19) (eupnea and artificial reflexogenic hyperpnea). Antagonism was greater to extrinsic than to intrinsic acetylcholine.

It is thought that the support which our experiments give to the "humoro-electrical" theory of nerve cell function may prove basic to neurophysiological concepts.[2]

[2] Since our paper has gone to press highly significant data have appeared on synaptic and motor end plate potentials. Repetitive stimulation of preganglionic fibers (low frequency) produces individually independent synaptic potentials. With greater frequencies there is a stepped rise to a wavy plateau. This plateau is attained earlier and is higher the higher the frequency. Undulations corresponding to stimulation may disappear entirely. (See Eccles, J. P. 1943, **101**, 465.) Such smoothed synaptic potentials would seem to correspond with the requirement of our concepts that pooling of acetylcholine provides an adjustable continuous current for activating the neuraxon as it leaves the axon hillock (see p. 383).

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THE OCCURRENCE OF A VASOCONSTRICTOR SUBSTANCE IN BLOOD DURING SHOCK INDUCED BY TRAUMA, HEMORRHAGE AND BURNS

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Shock elicited by the tourniquet method and by hemorrhage is associated with arterial and arteriolar vasoconstriction until death of the animal approaches. Doctor Abell and I (1) could show this objectively by observing the vessels in windows made according to the Clark principle. These had been placed in the ears and mesenteries of rabbits and the mesenteries of cats. The mechanism by which this vasoconstriction occurs is not clear, but the fact that it is so persistent suggests that, in part, it is due to continuous liberation of a vasoconstrictor substance.

For this reason it seemed desirable to ascertain whether such a substance could be demonstrated in the blood of animals in shock induced by a variety of means. The perfused rabbit's ear method (2) was employed for demonstrating the presence of vasoconstrictor substance in dog's blood. Shock was induced by *1*, tourniquets applied to the limbs; *2*, severe hemorrhage; *3*, stripping of the intestines, and *4*, burns.

METHODS. Shock was produced in dogs under pentobarbital anesthesia (35 mgm./kgm. body weight) first by tourniquets placed around the hind limbs of dogs. The tourniquets were sufficiently tight to reduce arterial pressure distal to the obstruction to 30 mm. Hg or less and all return flow was not cut off. They were left in place for $3\frac{1}{2}$ to $4\frac{1}{2}$ hours. Blood samples were taken into syringes moistened with sodium citrate solution and coagulation was prevented by addition of 0.3 cc. of 10 per cent sodium citrate to every 5 cc. of blood. Shock was also produced by bleeding dogs up to as much as 7 per cent of their body weight and keeping the arterial pressure at 30 mm. Hg for 40 minutes. The details of this method will be described by Kohlstaedt and Page (3).

Stripping and exposure of the intestine has been used to produce shock. Dr. Robert Taylor, who is studying this method, supplied the blood samples. Shock was also produced by burning the extremities of anesthetized dogs by immersion in boiling water for from 20 seconds to 3 minutes.

Samples of citrated plasma were perfused through the isolated rabbit's ears using either calcium-free Ringer's solution or citrated normal dog's plasma as perfusing medium. Perfusion pressure may be kept very low (10/0 mm. Hg) when citrated plasma is used instead of defibrinated blood. Vasoconstriction in the ear was measured by the length of time required for the drop rate to be restored to its initial value as measured before the test injection, and by the

percentage reduction of drop rate after the injection as compared with the rate before.

All of the plasma samples (0.1 to 0.2 cc.) to be tested were first injected into the ear perfused with calcium-free Ringer's solution. After the intensity of the vasoconstriction had been thus ascertained, citrated plasma from normotensive dogs was substituted for the calcium-free Ringer's solution and the plasma samples again tested. This was done because at least two kinds of vasoconstrictor substances appear in blood. One occurs after blood has clotted (4) and causes vasoconstriction in the ear when either Ringer's solution or plasma perfuses it. The other, which occurs in blood of hypertensive patients and dogs, causes significant vasoconstriction only when plasma or blood are the perfusing media (5).

RESULTS. 1. *Tourniquet shock.* Blood samples from 32 dogs in shock have been studied. They were taken within 20 minutes after the tourniquets were in place and at intervals for several hours.

Plasma from these animals caused vasoconstriction within ½ hour after applying the tourniquets both with calcium-free Ringer's solution and plasma as perfusing media (table 1). The amount of vasoconstrictor appeared to increase during the first hour but from then until death of the animal—usually from one to five hours after release of the tourniquets—no further increase occurred. No relationship was found to the level of arterial pressure.

Seven additional experiments were performed in which both kidneys were removed the day before tourniquet shock was induced (example in table 1). The vasoconstrictor appeared in the blood just as it had in intact animals. Three experiments were then done in which the left renal pedicle was denervated and the right kidney removed three days before the experiment. Again application of tourniquets and production of shock caused the appearance of the vasoconstrictor. Even section and partial destruction of the spinal cord from the second thoracic segment caudad failed to prevent its appearance when shock occurred.

2. *Hemorrhage.* From 2 to 7 per cent of the body weight of blood of the dog was removed rapidly. Arterial pressure usually fell to 30 to 40 mm. Hg but tended to rise if more blood was not removed. Within 5 minutes after such a severe hemorrhage, vasoconstrictor was found in the blood (table 2). It persisted throughout the period of hypotension. We have not determined how long it remains after blood pressure was restored by transfusion. Eighteen animals were used for these experiments.

In 5 additional dogs bilateral nephrectomy was performed varying from one day to one hour before bleeding. Vasoconstrictor appeared in the blood as in intact dogs.

Six experiments were also done in which both adrenal glands were removed from 1 day to 3 hours before the experiment. Due to susceptibility of these animals to vasomotor collapse it was necessary to bleed them more cautiously than the normal dogs. The operation did not interfere with the appearance of the vasoconstrictor substance in the blood.

TABLE 1

Formation of vasoconstrictor as a result of shock produced by constricting the blood supply to limbs of dogs

DOG NO.	B.P.	HEMATOCRIT	TIME	PROCEDURES	PLASMA PERFUSED		RINGER'S SOLUTION PERFUSED	
					Reduction of flow	Per cent reduction of drop rate	Reduction of flow	Per cent reduction of drop rate
	mm. Hg				*minutes*		*minutes*	
16-22	234	49	8:30	Control	½	20	0	0
			8:40	Cords on legs				
	142		12:15	Released				
	122	75	3:10					
	130	73	4:00		7	59	6	44
	110	73	6:00		6	31		
	86	70	7:10	Died 6 hrs. later	19	49	7	70
16-24	165	37	4:30		0	0	1½	22
			4:45	Cords on legs				
	124	56	7:00		9	45		
	104	56	7:55		12	59		
			8:00	Released				
	66	61	8:45		12	54	6	68
	56	62	9:06	Died 1 hr. later	7½	40		
16-12	190	50	8:00	Bilateral nephrectomy 1 day before	2½	54	0	0
			8:10	Cords on legs				
	130	54	10:00		9	60	3	45
			11:05	Released	9	70	3	48
	62	59	11:15		9+	75	4	50
	58	60	1:00					
			1:30	Died suddenly				
16-46	148	40	7:10	Left renal denervation and right nephrectomy 3 days before	1	23	1½	36
			7:15	Cords on legs				
	124	62	9:15		2½	38		
	134	62	10:50		2½	36		
			11:00	Released				
	108	67	1:20		3	30		
	92	67	4:15		3	27		
	64	65	6:05		5	30	4½	60
16-43	122	42	11:30		0	0		
	44		12:00	Animal pithed				
			12:15	Cords on legs				
	44	47	1:30		4½	56		
	46	47	3:05					
	34		3:15	Cords released				
	32	51	3:40		2½	46		
	22	51	5:10		2½	47		

TABLE 2

The effect of hemorrhage in normal and nephrectomized dogs on the formation of vasoconstrictor

ORIGIN OF BLOOD	MEAN ARTERIAL PRESSURE	PLASMA PERFUSED		RINGER'S SOLUTION PERFUSED	
		Reduction of flow	Per cent reduction of drop rate	Reduction of flow	Per cent reduction of drop rate
	mm. Hg	*minutes*		*minutes*	
Normal dog					
Control	160	1½	25	½	10
2 min. after 7.6 cc./kgm. blood removed	40	¼	14		
1½ hours later	42	3¾	44	5¼	36
10 min. after 3 cc./kgm. blood removed	30	2	33		
10 min. after 3 cc./kgm. blood removed	34	5½	46	5¾	83
10 min. after 3 cc./kgm. blood removed	32	6½	48		
Same sample		5¾	38		
Next day:					
Control	110	2	38	1	32
1½ hrs. after 3 cc./kgm. blood removed	42	5	50	3	63
2½ hrs. later	46	2½	36		
¾ hr. after 3 cc./kgm. blood removed	24	5½	46		
1¾ hrs. after 3 cc./kgm. blood removed	20	11	60		
Normal dog					
Control	136	0	0	¾	34
200 cc. blood removed and sample taken 45 minutes later	56	5¼	43	6½	88
Normal dog					
Control	128	½	20	1½	22
200 cc. blood removed; sample taken immediately	78	¾	12		
3 hours later	40	3¼	42	2¼	64
Nephrectomized dog (24 hours before)					
Control	142	1¼	28	0	0
1 hr. after 4 cc./kgm. blood removed	80	2	30	2	30
1 hr. after 4 cc./kgm. blood removed	72	2	55		
½ hr. later	60	1½	27		
1½ hrs. later	78	1¼	7		
½ hr. later	46	3½	48	3¼	38
¼ hr. later	38	2¼	50		
Nephrectomized dog (3 hours before)					
Control	130	1	19	1¼	32
Bled 3 per cent of body weight—20 minutes after bleeding	42	4	47	3½	44
42 min. after bleeding	24	6	70		
68 min. after bleeding	50	2¼	47		
90 min. after bleeding	100				
All blood returned					
105 min. after bleeding	84	0	0		

Apparently adrenalin is either not liberated in large amounts or the method is too insensitive to detect small changes in it when hemorrhage occurs. This observation was unexpected since we had thought adrenalectomy might reduce the amount of vasoconstrictor found after hemorrhage.

3. *Intestinal stripping.* The results with this method for producing shock were the same as with the tourniquet method. Before the arterial pressure fell significantly, the plasma developed vasoconstrictor properties which appeared to persist until death of the animal. Four experiments of this type were performed (table 3). Ultra-filtrates prepared from citrated plasma produced as much vasoconstriction as the original plasma from which it was prepared.

4. *Burns.* Within 5 minutes after severe burns of the extremities from boiling water, the citrated plasma of the dogs showed marked hemoglobinemia. Vasoconstrictor action of the plasma was evident when perfused with calcium-free Ringer's solution through a rabbit's ear. Twenty-four experiments were done (table 3).

Since so much hemolysis had occurred as the result of the burns, it seemed desirable to ascertain how much this might contribute to the vasoconstrictor action of the plasma. Washed, packed red blood cells were laked with distilled water, the stroma removed by centrifuging, the supernatant solution added to plasma until the color matched to the naked eye that of the plasma from burned dogs. Such plasma caused slightly more vasoconstriction than did the control normal plasma but nothing like enough to account for the vasoconstrictor occurring in the plasma of burned animals.

Plasma from burned animals was subjected to ultra-filtration. The protein-free filtrate in quantities of 0.2 cc. caused usually as much vasoconstriction as did the original plasma. The constriction was more prolonged but seldom as sharp as that caused by the plasma itself.

For some unexplained reason several ultra-filtrates from plasma of burned animals showed no vasoconstrictor action. This was also true of serum, hence we are of the opinion that some uncontrolled factor affected the results. Since the majority of the ultra-filtrates showed marked vasoconstriction we are inclined to view the results as indicating that the vasoconstrictor is ultra-filtrable. This is strengthened by the fact that dialysis of plasma from burned dogs and normal serum for 17 hours in cellophane against normal salt solution causes disappearance of the vasoconstrictor action of the plasma or serum left within the dialysing sac (3 expts.).

If the ultra-filtrate was subjected to heat by immersing the tube containing it for 5 minutes in boiling water, the vasoconstrictor action was reduced but not abolished. About the same reduction in potency occurred when plasma was treated similarly, and after boiling, the coagulum filtered off. The vasoconstrictor substance is evidently not highly thermolabile.

5. *Dissimilarity of the vasoconstrictors occurring in plasma from shocked and burned dogs from those in hypertensives and in serum.* It has been demonstrated (5) that plasma and blood of hypertensive patients and dogs contains a vasoconstrictor substance as shown by the fact that they cause vasoconstriction

when perfused with normal plasma or blood through rabbits' ears. When Ringer's solution was used, very little vasoconstriction resulted. This fact aids

TABLE 3

Effect of burns and intestinal stripping in dogs on occurrence of vasoconstrictor in plasma (0.2 cc.) and plasma ultra-filtrates (0.2 cc.)

DOG NO.	TIME	PROCEDURES	PERFUSION MEDIUM	VASOCONSTRICTION IN RABBIT'S EAR	
				Reduction in drop rate	Per cent reduction of drop rate
				minutes	
19-25	Control	Anesthetized	Ringer's*	¼	16
	5 min.	After burn	Ringer's	16	75
	1 hour	After burn	Ringer's	4¼	79
	Control		Plasma	2	17
	5 min.	After burn	Plasma	8¾	81
	1 hour	After burn	Plasma	11½	82
19-23	Control	Anesthetized	Ringer's	1¼	34
		Intestine stripped	Ringer's	6½	85
		Ultra-filtrate	Ringer's	8¾	52
		Boiled ultra-filtrate	Ringer's	½	34
19-34	Control	Anesthetized	Ringer's	1½	23
	30 min.	After burning	Ringer's	6½	55
		Plasma heated in boiling water for 5 minutes	Ringer's	2½	35
		Ultra-filtrate	Ringer's	4½	59
		Plasma heated in boiling water for 5 minutes	Ringer's	3½	47
19-43	Control	Anesthetized	Ringer's	1¼	30
	10 min.	After burn	Ringer's	7	98
	1 hour	After burn	Ringer's	7¾	93
		Boiled 5 minutes	Ringer's	3½	81
		Ultra-filtrate	Ringer's	15	56
		Ultra-filtrate in boiling water-bath, 5 minutes	Ringer's	4¼	48
		Ultra filtrate	Ringer's	5	38
	2nd Assay		Ringer's		
	Control	Anesthetized	Ringer's	1	22
	1 hour	After burn. Plasma ultra-filtrate	Ringer's	10½	100
		After burn. Plasma	Ringer's	8¾	81
		Ultra-filtrate in boiling water-bath 5 minutes	Ringer's	3	55

* Calcium free.

in distinguishing the vasoconstrictor action of hypertensive's plasma from plasma drawn from shocked and burned dogs. But normal *serum* alone also causes vasoconstriction in the rabbit's ear when calcium-free Ringer's solution

perfuses the ear just as does plasma from shocked and burned animals. Confusion with histamine might also occur because it causes vasoconstriction under the conditions of our experiment. It was, therefore, desirable to determine whether the same vasoconstrictor occurred in blood of shocked and burned animals as that formed when blood coagulates. Further, it was necessary to distinguish these from histamine.

It was found that repeated injections of one type or another of plasma or serum ultimately causes the ear vessels of an occasional preparation to become refractory to further stimulation to this especial plasma or serum. Let us take, for example, the continued perfusion and injection of dog's serum until vasoconstriction no longer results. If now plasma from a burned dog was injected the full vasoconstrictor response was elicited. This principle was used to ascertain the similarity or dissimilarity of the various vasoconstrictors described in this investigation.

The rapidity with which refractoriness developed in different ears varied greatly. It seemed usually much easier to develop it in an ear perfused with plasma when hypertensive plasma was used than when normal serum was employed and the ear perfused with calcium-free Ringer's solution. As many as 60 injections of serum often failed to produce complete refractoriness in some ears. In order to facilitate its development, the experiments were conducted by perfusing for 30 minutes to an hour some of the plasma or serum to which refractoriness was desired, along with calcium-free Ringer's solution or normal citrated plasma. The experiments illustrated in the tables are those in which refractoriness developed quickly.

Refractoriness elicited to dog's serum abolished the vasoconstrictor action of human serum as well (table 4). But plasma from burned dogs and dogs in shock from tourniquets or hemorrhage still caused sharp vasoconstriction. Histamine also was active. It therefore appears that the vasoconstrictor in plasma of burned, bled, or shocked animals is not identical with that in dog or human serum. The vasoconstrictors in dog and human serum appear from this test to be identical. Histamine differs from the latter in that it continued to cause vasoconstriction after the ear was refractory to serum.

The next step was to perfuse ears with plasma from burned dogs and establish refractoriness to it (table 4). Human serum now gave full response while plasma from bled dogs gave none. Histamine was still active. These experiments, of which there were two, show again that the vasoconstrictor in burned animals differs from that in serum and that histamine is unlike both.

To show that the vasoconstrictor in hypertensive's blood differs from that in both shocked dog's plasma and in serum, ears were perfused with hypertensive dog's plasma until refractoriness developed. Repeated injections of human hypertensive's plasma was used to hasten it and also to ascertain the rate of development. When the ear no longer responded to either hypertensive human or dog's plasma, burned and bled dog's plasma still caused vasoconstriction (table 5). The same ear was then perfused with normal dog's plasma plus burned dog's plasma. Refractoriness to burned dog's plasma developed but now hypertensive's plasma again caused vasoconstriction. Human serum also

TABLE 4

Development of refractoriness to dog's serum and burned dog's plasma and the relationship to the vasoconstrictor response to plasma of burned dogs in hemorrhagic shock and histamine

SUBSTANCE INJECTED	AMOUNT	DROP RATE PER MINUTE	REDUCTION OF DROP RATE	PER CENT REDUCTION OF FLOW
	cc.		*minutes*	
(1) Ear perfused with calcium-free Ringer's solution plus dog's serum (0.15 cc. serum/cc. calcium-free Ringer's solution). Perfusion pressure 44/24 mm. Hg				
Dog serum	0.2	48	3½	42
Dog serum	0.2	48	1	25
Dog serum	0.2	48	1	31
Human serum	0.2	40	1¼	22
Human serum	0.2	44	0	0
Burned dog plasma	0.2	44	8	82
Human serum	0.2	48	0	0
Human serum	0.2	48	0	0
Burned dog plasma	0.2	48	6	76
Human serum	0.2	60	0	0
Histamine (0.000027 mgm./cc.)	0.2	60	4+	100
Human serum	0.2	60	½	33
Human serum	0.2	64	½	6
Dog plasma from hemorrhagic shock	0.2	64	2	55
Human serum	0.2	60	0	0
Histamine (0.000027 mgm./cc.)	0.2	60	3¼	72
(2) Second ear perfused with calcium-free Ringer's solution for 30 minutes. Perfusion pressure 40/24 mm. Hg				
Human serum	0.2	44	2½	47
Burned dog plasma	0.2	44	4½	56
Dog plasma from hemorrhagic shock	0.2	48	6	77
Histamine (0.000027 mgm./cc.)	0.2	60	6	33
Human serum	0.2	52	2¾	51
(3) Ear perfused with plasma from burned dog (0.25 cc. plasma/cc. calcium-free Ringer's solution). Perfusion pressure 64/46 mm. Hg				
Burned dog plasma	0.2	44	3	55
Burned dog plasma	0.2	44	2½	59
Burned dog plasma	0.2	40	2½	—
Burned dog plasma	0.2	36	2	—
Burned dog plasma	0.2	36	1	—*
Burned dog plasma	0.2	36	1	27
Burned dog plasma	0.2	36	1	22
Burned dog plasma	0.2	44	0	0
Human serum	0.2	36	3	46
Dog plasma from hemorrhagic shock	0.2	40	0	0
Histamine (0.000027 mgm./cc.)	0.2	40	4¼	59
Burned dog plasma	0.2	36	¼	11
Dog plasma from hemorrhagic shock	0.2	36	0	0
Human serum	0.2	36	2	31

* Given in rapid succession

caused vasoconstriction. These results suggest that the vasoconstrictors in dog and human hypertensives are similar but differ from those in burned or bled dog's plasma.

TABLE 5

Development of refractoriness to plasma of hypertensive patients and the relationship to the vasoconstrictor response to plasma of burned dogs and dogs with hemorrhagic shock

SUBSTANCE INJECTED	AMOUNT	DROP RATE PER MINUTE	VASOCONSTRICTION IN RABBIT'S EAR	
			Reduction of drop rate	Per cent reduction of flow
(1) Ear perfused with calcium-free Ringer's solution for 15 minutes				
	cc.		*minutes*	
Burned dog's plasma	0.2	60	3	57
(2) Ear perfused with hypertensive dog's plasma (diluted 25 per cent with calcium-free Ringer's solution). Perfusion pressure 40/20 mm. Hg				
Human hypertensive's plasma	0.2	13	4	35
Human hypertensive's plasma	0.2	17	1	24
Human hypertensive's plasma	0.2	17	1	17
Human hypertensive's plasma	0.2	15	2	50
Hypertensive dog's plasma (another dog)	0.2	13	1¾	23
Hypertensive dog's plasma	0.2	15	1¾	28
Hypertensive dog's plasma	0.2	15	¼	12
Human hypertensive's plasma	0.2	15	0	0
Burned dog's plasma	0.2	15	6+	100
Hypertensive dog's plasma	0.2	13	0	0
Human hypertensive's plasma	0.2	15	0	0
Burned dog's plasma	0.2	17	7	72
Hypertensive dog's plasma	0.2	13	0	0
Plasma from dog in hemorrhagic shock	0.2	13	3½	45
(3) Ear perfused with normal dog's plasma (9 parts) plus burned dog's plasma (1 part) 2 hours after last injection. Perfusion pressure 48/24 mm. Hg				
Burned dog's plasma	0.2	13	5	61
Burned dog's plasma	0.2	11	5	33
Burned dog's plasma	0.2	13	1	8
Hypertensive dog's plasma	0.2	11	5	39
Burned dog's plasma	0.2	13	0	0
Hypertensive dog's plasma	0.2	13	4	25
Burned dog's plasma	0.2	13	½	13
Human serum	0.2	13	3½	58

6. *Use of the intestinal strip method.* Because of some uncertainty in the method just described, it seemed desirable to obtain confirmatory evidence using another method. The isolated rabbit's intestinal ring preparation was selected.

Rings from 2 to 3 cm. in length were cut from the small intestine and kept

in Ringer's solution in the ice box until ready for use. The temperature of the 30 cc. cups were thermostatically controlled to within 0.1°C. Glucose containing, but calcium-free Ringer's solution, was used as the bathing medium. When a stable rhythm was established the test substance was added in 1 cc. doses.

The addition of dog or human serum causes sharp contraction of the gut while citrated plasma causes none in the doses employed. Citrated plasma from blood of burned dogs or dogs subjected to tourniquet shock caused no contraction. The same plasma had been tested on the perfused rabbit's ear and shown to cause marked vasoconstriction. These experiments, of which 9 were done, show that the vasoconstrictor in serum differs from that in plasma from burned and shocked animals.

Discussion. The purpose of this investigation was to learn more about the mechanism by which vasoconstriction was induced when animals were in secondary shock, whether from tissue injury, hemorrhage or burns. It is apparent that plasma from animals in, or approaching shock produced by several methods, acquires vasoconstrictor properties as measured in the isolated perfused rabbit's ear.

The chemical nature of the vasoconstrictor is not known. Since it causes vasoconstriction when the ear is perfused with Ringer's solution it is unlikely that it is identical with the vasoconstrictor which occurs in experimental or clinical renal hypertension. It is probably ultra-filtrable and hence it might be assumed to be a crystalloid of not excessively high molecular weight. It is also not markedly heat-labile.

These properties are not unlike those of the vasoconstrictor substance which results when normal blood coagulates (4–6). The serum formed causes much greater vasoconstriction in the rabbit's ear than does the plasma.[1] Furthermore, vasoconstriction occurs when Ringer's solution is the perfusing medium, thus differentiating it from the vasoconstrictor of hypertensives whose action is dependent upon the presence of plasma or blood. The vasoconstrictor of serum can also be separated by ultra-filtration (5), an observation which we can confirm.

It is possible that the vasoconstrictor which occurs in shock, and that occurring when blood coagulates, are similar. In view of the widespread tissue destruction which occurs in burns, after application of tourniquets and after stripping of the intestine, it would not be surprising that chemical changes associated with coagulation might be initiated.

Another possibility should be considered; namely, that the vasoconstrictor is histamine. It is recognized that histamine can cause vasoconstriction in the vessels of the ears of intact rabbits (7, 8, 9) and we have found it to act similarly in the perfused ears under the conditions of our experiments.

Neither of these hypotheses receives any support from the experiments in

[1] In a previous paper results were published which suggested that plasma and serum were equal in vasoconstrictor properties. This was an unfortunate mistake because many results both before and after showed the contrary, as, indeed, did the results in the literature. Dr. Eugene Landis was good enough to call our attention to this error.

which refractoriness was used as a method to identify a particular vasoconstrictor. This is an interesting method but one which has not as yet been intensively studied to ascertain its mechanism. It appears to depend on the fatiguing of certain elements in the vascular musculature by repeated injection of, and perfusion with, a particular plasma or serum. When the vessels no longer respond by vasoconstriction, the plasma or serum with which it is to be compared is injected. When no response occurs, it is taken as evidence in favor of similarity to the fatigue inducing plasma or serum. Sharp vasoconstriction, on the contrary, suggests dissimilarity.

It is worth disgressing to point out that the development of refractoriness occurs at unusually different rates. Some ears continue to respond by vasoconstriction while perfusing with the fatigue-producing serum or plasma and after the injection of 60 or more samples of serum, while in a rare one refractoriness is elicited after several injections. The latter experiments seem to be the ones suited to demonstrate the similarity or dissimilarity of vasoconstrictors.

The importance of the method is greatly weakened by the fact that only certain ears cease to respond to the repeated stimulus readily and we have been unable to determine why this is so. It is thus not an easily reproducible phenomenon and correspondingly cannot be given as much weight as we might like. Nevertheless, when refractoriness develops, the results are so clear they can hardly be doubted. These reservations must be kept clearly in mind in the interpretation of the results pertaining to the dissimilarity of the vasoconstrictors in serum and shock plasma.

Whether this method gives proof of identity seems uncertain. It is conceivable that the vasoconstrictor could be the same but the quality or quantity of another substance present in plasma could so modify the vasoconstrictor action as to give misleading conclusions. Several years ago we showed (10) that in the perfused ear, plasma or blood was necessary for angiotonin to exert its full vasoconstrictor action. We postulated the presence of an "angiotonin-activator" in blood. This substance might have a potent orienting influence on the vasoconstrictor. It seems more reasonable, however, to assume that vasoconstriction can be elicited by a variety of mechanisms one or more of which may be fatigued by repeated stimuli. The fatigue of one does not preclude vasoconstriction occurring as a result of the action of another. It is this type of mechanism with which we appear to be dealing in these experiments.

The results of application of the method leave little doubt that, plasma from traumatized, bled and burned dogs contain similar vasoconstrictors which differ from that formed when blood coagulates. Both of these differ from that present in the plasma of chronic hypertensives. Furthermore, histamine differs from all of them. The only question is whether this method is a valid one.

Some support is offered by the work on intestinal strips. These respond regularly by severe contraction to serum but plasma causes little or none. Histamine also causes contraction. But plasma from burned or shocked animals does not cause any more contraction than plasma of normal dogs. The same plasma which elicited sharp constriction in the rabbit's ear was inactive on intestine.

Neither the kidneys nor the adrenal glands seem necessary for the formation of the vasoconstrictor for it occurs in their absence when shock is produced by hemorrhage and tourniquets. Nephrectomy, renal denervation, and low spinal cord destruction do not prevent its appearance in tourniquet shock. It seems not unreasonable to suppose that the occurrence of a vasoconstrictor arising in shock is associated with anoxia of the tissues, since this is at least one of the factors common to shock produced by these different methods.

It is of great interest that Sapirstein, Ogden and Southard (11, 12) have presented evidence that hemorrhage causes the renin content of the blood to increase; possibly as a "homeostatic" effort on the part of the body to restore blood pressure to normal levels. Hamilton and Collins (13) have shown that hemorrhage imparts *pressor* activity to blood as tested on nephrectomized dogs. This occurs in normal and adrenalectomized animals as well as in those with the kidneys denervated. The type of pressor response was suggestive of the presence of both renin and angiotonin. The kidneys were the source of these substances because *1*, renal vein blood usually gave greater responses than the corresponding arterial blood, and *2*, pressor reactions were obtained from the blood of adrenalectomized dogs, but not from dogs with both kidneys and adrenal glands removed. Huidobro and Braun-Menendez (14) also found renin liberated by hemorrhage in intact anesthetized dogs. Renin could be detected in the systemic blood of these dogs but not in nephrectomized animals after hemorrhage.

These results, in conjunction with our own, suggest that at least two substances are involved in hemorrhagic shock, one from the kidneys having pressor qualities and the other from elsewhere in the body having vasoconstrictor properties.

SUMMARY. 1. Shock has been produced in dogs by means of *a*, tourniquets placed around the hind extremities; *b*, stripping and exposure of the intestines; *c*, hemorrhage; *d*, burns.

2. Vasoconstrictor properties develop in the blood of such animals which can be demonstrated by perfusing samples of citrated plasma through isolated perfused rabbits' ears. Vasoconstriction results regardless of whether the ears are being perfused with calcium-free Ringer's solution or by citrated plasma from normal dogs.

3. Renal denervation, nephrectomy, adrenalectomy and low spinal destruction do not prevent the appearance of vasoconstrictor after tourniquet shock. Nor does nephrectomy and adrenalectomy prevent it after hemorrhage.

4. Refractoriness to the vasoconstrictor action of sera and plasma may be produced by perfusion with, and repeated injections of them. Once established, the vessels of the rabbit's ear still respond to plasma of different origin. This method has been used to ascertain similarity or dissimilarity among the various plasma and sera containing vasoconstrictors.

5. Refractoriness to serum does not abolish the vasoconstrictor action of plasma from burned, bled or shocked dogs, or to histamine. Refractoriness to plasma from burned dogs does not abolish the vasoconstrictor action of serum or histamine but does do so to plasma from bled or hypertensive dogs. Re-

fractoriness to hypertensive's plasma does not abolish the vasoconstrictor action of burned or bled dog's plasma.

6. Rabbits' isolated intestinal rings contract powerfully when serum is added to calcium-free Ringer's solution bathing them. Normal citrated plasma and plasma from burned or shocked dogs causes no contraction in dilutions of 1 to 30.

CONCLUSIONS

Shock, whether elicited by tourniquets placed around the extremities, stripping and exposing the intestines, hemorrhage and burns, is associated with the appearance in the plasma of a substance which causes vasoconstriction in rabbits' ears perfused with either calcium-free Ringer's solution or plasma. It does not originate in the kidneys, adrenal glands, nor does destruction of the spinal cord or renal denervation prevent its appearance. Evidence gathered from application of a method depending on "fatiguing" the vascular musculature suggests, if the validity of the method is acceptable, that the vasoconstrictor action of plasma from burned, bled and shocked dogs is caused by identical or very similar substances. Furthermore, it differs from the vasoconstrictors present in hypertensive's (human and canine) plasma and in serum (human and canine). None of these vasoconstrictors seem to be histamine. This belief is confirmed by experiments on isolated intestine which show that serum causes marked contraction while plasma from burned or shocked dogs causes none.

I am grateful to Mr. John Tilden for his skilled technical assistance.

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EFFECT OF COBALT ON WORK PERFORMANCE UNDER CONDITIONS OF ANOXIA[1]

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In an earlier report a method for measuring the work performance of rats under conditions of anoxia was described in detail (1). This method consisted, essentially, of exercising rats to the endpoint of exhaustion in circular, motor driven cages in an atmosphere of 6.8 per cent oxygen (52 mm. Hg partial pressure).

The development of polycythemia in animals and humans living at high altitude has long been regarded as an important factor in successful adaptation to such an environment. Recent studies indicated that administration of cobaltous ion in small doses induced polycythemia (2, 3, 4, 5, 6). It seemed worthwhile, therefore, to determine if the polycythemia induced by cobalt administration facilitated work performance under conditions of reduced oxygen tension.

METHODS. In these experiments, male rats of the McCollum strain were maintained on a constant synthetic diet which contained adequate quantities of minerals and vitamins. The method of conducting work performance tests under conditions of reduced oxygen tension has been described (1). After a suitable training period ten animals were selected on the basis of reproducibility of performance. Cobalt ($COCl_2 \cdot 6H_2O$) was given in the drinking water to five of these animals. The dose of cobalt was so adjusted that each cubic centimeter of drinking water contained 100 gamma of cobaltous ions. Cobalt administration was continued by this method for fourteen weeks, except for a period from the second to fourth weeks during which 0.6 mgm. of cobaltous ions in 0.1 cc. of normal saline was injected subcutaneously daily. During this period, tap water was substituted for the cobalt solution. The remaining five animals drank tap water during the entire experiment and were used as controls.

Measurements of work performance, in an atmosphere consisting of 6.8 per cent oxygen (52 mm. Hg partial pressure) and 93.2 per cent nitrogen, were made at appropriate intervals during the period of cobalt administration and for six

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Harvard University. The pathologic studies have been aided by a grant to the Department of Pathology from the Josiah Macy Jr. Foundation.

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weeks after cobalt was discontinued. In these experiments it was found advantageous to observe the animals during work performance tests for five minutes after they appeared exhausted, rather than for two minutes as suggested in an earlier report (1). The five trained animals which were used as controls for the work experiments were always tested at the same time as the cobalt treated animals.

Two additional groups of nine animals each were used as controls. One group received cobalt without work performance tests. Cobalt treatment in this group differed slightly from that in the work performance test group in that oral administration was used exclusively except during the first week. The remaining group received neither cobalt nor work performance tests.

Hemoglobin was determined as acid hematin by means of a photoelectric colorimeter. Blood specimens for these determinations were obtained from the cut surface of the tip of the tail.

Complete autopsies were performed on all animals. The two groups of animals which underwent work performance tests were sacrificed six weeks follow-

TABLE 1

Effect of cobalt treatment on hemoglobin values of rats

GROUP	DURATION OF COBALT TREATMENT				
	2 weeks	6 weeks	8 weeks	11 weeks	14 weeks
Work performance test (anoxia) plus cobalt (5 animals)	10.9	20.4	21.3	20.9	21.6
Work performance test (anoxia) alone (5 animals)		16.3	14.8	15.8	15.5
Cobalt alone (9 animals)	16.7	16.8	19.1	20.5	
Controls (9 animals)	14.9	15.4	15.6	15.8	

ing withdrawal of cobalt. The animals which did not participate in work performance studies were sacrificed immediately following eleven weeks of cobalt, at a time when their hemoglobin response was at its maximum.

OBSERVATIONS. *Effect of cobalt on hemoglobin values.* As indicated by earlier workers, administration of cobalt to normal rats was followed by a striking polycythemia (table 1). After five to six weeks of cobalt the hemoglobin of the five treated animals (work performance test group) rose from an average initial value of 15.6 grams per 100 cc. of blood to 20.4 grams per 100 cc. of blood. During this period there was no significant change in the hemoglobin value of control rats (work performance test group). In the group of nine animals treated with cobalt but not given work performance tests, hemoglobin values rose from an average level of 16.7 grams to 19.1 grams after eight weeks of cobalt (table 1). It is probable that the longer period of cobalt administered by the parenteral route in the work performance test group was responsible for the more rapid development of polycythemia in this group of animals. Both groups of animals, however, ultimately attained the same hemoglobin levels at the end of eleven weeks of treatment.

Polycythemia persisted as long as cobalt was continued in all of the animals except one. This animal's hemoglobin value fell to 13.8 grams during the eleventh week of cobalt treatment after having attained the high value of 19.0 grams during the eighth week.

Following cessation of cobalt, the polycythemia gradually subsided. A group of five rats with average hemoglobin values of 21.6 grams per 100 cc. at the end of fourteen weeks of cobalt administration showed average hemoglobin values of 18.1 grams three weeks after cobalt was discontinued (table 2).

Effect of cobalt on body weight. During fourteen weeks of cobalt administration the five work performance test animals gained an average of 82 grams, whereas the five work performance test animals which did not receive cobalt gained an average of 117 grams. Following cessation of cobalt treatment, the work performance test group displayed an accelerated rate of weight gain which enabled them within a period of six weeks to equal the controls in weight. No alteration in the rate of

TABLE 2

Changes in hemoglobin values following withdrawal of cobalt

ANIMAL	AT END OF 14 WEEKS OF COBALT TREATMENT	FOLLOWING WITHDRAWAL OF COBALT TREATMENT		
		1 week	2 weeks	3 weeks
1	23.0	22.2	22.0	20.4
2	23.5	21.6	18.8	18.0
3	22.0	20.2	15.0	18.6
4	20.6	20.0	17.5	18.6
5	19.0	19.0	18.9	14.9
Mean	21.6	20.6	18.4	18.1

growth was observed between the cobalt treated controls (not given work performance test) and the totally untreated group. These data indicate that with this dose of cobalt, retardation in growth did not occur unless an added stress (work performance test in anoxia) was present. Treatment with larger doses of cobalt alone or exposure for longer periods to anoxia alone retarded growth.

Effect of cobalt on work performance under conditions of anoxia. On two successive tests of base-line performance five animals selected for cobalt treatment averaged 11.3 and 12.2 minutes respectively, and the control animals averaged 9.2 and 11.0 minutes respectively (chart 1).

During the first four weeks of cobalt treatment the work performance of both cobalt treated and control animals decreased (chart 1). Coincident with the appearance of increased hemoglobin values (fifth to sixth week) work performance of the cobalt treated animals improved, reaching a maximum of 17.3 minutes during the seventh week of cobalt. This improvement in work performance of the group resulted from an increase in work performance of three of the five animals tested. Continuation of cobalt administration thereafter failed to

induce further improvement. In contrast, the control animals failed to exceed their base-line performances at any time during this fourteen-week period.

Shortly *after cessation* of cobalt treatment there was a striking increase in the work performance of *all* of the polycythemic rats. Measurements made during the first week after withdrawal of cobalt showed an average performance of 23.6 minutes, an improvement of 100 per cent over the mean base-line values. During this period the control animals showed no significant change in work performance.

The increased ability of the cobalt treated animals to perform work under conditions of anoxia did not appear to depend on cardiac hypertrophy since the heart weight in the two groups of animals was almost identical, i.e., 300 mgm./100 grams of animal in the cobalt treated group; 297 mgm./100 grams of animal in the

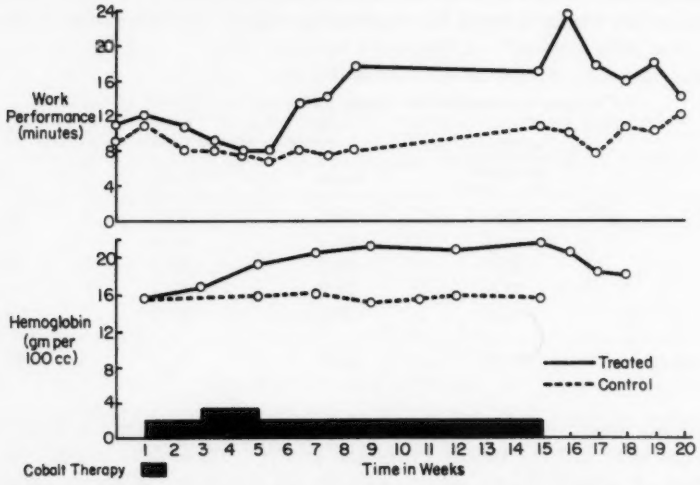

Chart 1. Effect of cobalt therapy on work performance and hemoglobin values of rats.

untreated control group. No significant difference in thymus weight was observed in the two groups of animals, i.e., 66 mgm./100 grams of animal in the cobalt treated group; 62.7 mgm./100 grams of animal in the control. There did appear to be a slight reduction in the weight of the adrenal glands in the cobalt treated animals, i.e., adrenal weight was 9.9 mgm./100 grams of animal in the cobalt treated group; 11.5 mgm./100 grams of animal in the control group. The cobalt treated group of animals weighed 344 grams (average), the control 351 grams (average).

Effect of cobalt on the hematopoietic system. The nine animals receiving cobalt but no work performance tests were autopsied immediately following eleven weeks of treatment. The only prominent gross finding was generalized vascular congestion of all tissues. There was slight enlargement of the spleen. The outstanding microscopic finding in these animals was a widespread increase in

erythropoietic activity. This was marked in the spleen (fig. 2), moderate in the bone marrow (fig. 4) and slight in the liver. The spleen showed depletion of the sheaths of lymphocytes that border the Malpighian corpuscles. In addition to these changes there was diffuse congestion of the lungs and kidneys. No evidences of injury, toxic or otherwise, were observed in the heart, liver and kidneys. In the control group of animals autopsied at the same time no evidence of abnormal or increased erythropoiesis was encountered (fig. 1 (spleen) and fig. 3 (bone marrow)).

All animals of the work performance groups were autopsied six weeks after the withdrawal of cobalt from the cobalt treated animals. Those which had received cobalt manifested hemosiderosis of the spleen, bone marrow and thymus, which may be considered to be indicative of an increase in blood destruction in these animals. No evidence of abnormal or increased erythropoesis was present in any organs of these animals. The spleens were almost twice as large as those encountered in any other group of rats in this study (fig. 5). Microscopically, this enlargement seemed to be due solely to vascular congestion. The Malpighian corpuscles were normal. Animals which had been tested for work performance under conditions of anoxia but had not undergone cobalt treatment showed no pathological differences from the control group not subjected to work performance tests.

Discussion. In these experiments the effect of cobalt in increasing the hemoglobin of normal animals was confirmed. The increased erythropoietic activity appeared to be most marked in the spleen, moderate in the bone marrow and slight in the liver. This is at variance with other reports (6, 7) which state that the spleen plays no essential rôle in this phenomenon. It is quite possible that this apparent discrepancy in observations may be explained on the basis of differences in dosage and duration of treatment. It is of interest to note that in one animal, the hematopoietic system was apparently depressed by cobalt after an initial stimulation. This inhibition of hematopoiesis resembled that recently noted in anemic dogs to whom cobalt had been administered (8).

Cobalt treated rats performing work under conditions of anoxia failed to gain weight as rapidly as control animals. Cobalt treatment alone did not influence the rate of growth of rats when the animals were not forced to perform work in an atmosphere of reduced oxygen tension. It is possible, therefore, that a latent toxicity of the cobalt ion was unmasked when animals treated with cobalt were subjected to repeated stress and strain.

An increase in the work performance in an atmosphere of reduced oxygen tension occurred in three of five cobalt treated rats, after polycythemia developed. When cobalt was withdrawn for a period too short to permit a significant fall in hemoglobin (1 wk.) a marked improvement in work performance of all five of the polycythemic animals occurred. It appeared probable, therefore, that cobalt influenced work performance favorably by inducing polycythemia, and unfavorably by some inhibiting effect. The improved performance achieved by animals under cobalt appears to be the resultant of these antagonistic actions. The striking increase in size of the spleen in rats subjected both to work per-

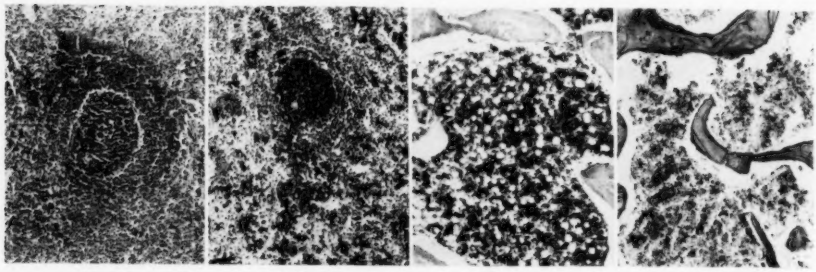

Fig. 1 Fig. 2 Fig. 3 Fig. 4

Fig. 1. Spleen of normal control rat.

Fig. 2. Spleen of rat subjected to cobalt treatment and to work performance tests, showing many islands of erythropoiesis in the red pulp, and lymphoid depletion, particularly around the follicles.

Fig. 3. Femoral marrow of normal control rat.

Fig. 4. Femoral marrow of rat subjected to cobalt treatment and to work performance tests, showing disappearance of fat and increase in foci of erythropoiesis.

All figures × 47, hematoxylin-eosin stain.

Fig. 5. Spleens from two rats representative of each group. Weights are given as recorded after fixation in formalin.

Top pair: cobalt and work performance tests; 1.01 grams, 1.28 grams.

Upper mid pair: cobalt only; 0.81 gram; 0.72 gram.

Lower mid pair: work performance tests only; 0.51 gram, 0.59 gram.

Bottom pair: normal control; 0.65 gram, 0.57 gram.

formance tests and to cobalt treatment is not open to simple interpretation, as the experiment included no rats allowed to recover from cobalt treatment without work tests.

CONCLUSIONS

1. Cobaltous ion in small doses induced erythropoietic activity in the spleen and liver and increased erythropoiesis in the bone marrow of normal rats. Significant increases in hemoglobin value were observed.

2. Animals so treated were observed to have an increased work performance under conditions of anoxia.

3. Although cobaltous ion in the dosage employed did not retard the growth of normal rats, some evidence of an untoward effect associated with its administration was indicated by the steady improvement in work performance which was noted immediately after withdrawal of cobalt and prior to the fall in hemoglobin values.

4. Enlargement of the spleen, apparently caused by vascular congestion, appeared in rats which were subjected to work performance tests and cobalt treatment and which were then allowed to continue six weeks after withdrawal of cobalt.

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THE INFLUENCE OF PREGNANCY, HYPERVITAMINOSIS-D AND PARTIAL NEPHRECTOMY ON THE VOLUME OF THE PARATHYROID GLANDS IN RATS

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A review of the literature indicates that three factors appear to be concerned with an increase in size and function of the parathyroid glands. That the glands undergo hyperplasia in chronic renal disease has been shown by Castleman and Mallory (1) on the basis of clinical material. A significant increase in glandular volume was demonstrated experimentally following partial nephrectomy in rats by Pappenheimer (2). Others have found that hyperparathyroidism, the functional counterpart of hyperplasia, exists in human beings with renal insufficiency as well as in dogs which have been subjected to experimental partial nephrectomy (3).

The relationship between pregnancy and the parathyroid glands is more obscure. It has been stated that hyperparathyroidism is three times more common in women and that it occurs with greatest frequency during the childbearing age (4). Evidence for a physiologic increase in parathyroid activity during normal gestation is clinical rather than experimental. Several workers appear to have demonstrated abnormally large amounts of parathyroid extract in the blood of pregnant women during the last trimester (5). Hyperplasia and increased vascularity of the glands during pregnancy have also been observed (6).

A third factor which is said to influence the size and function of the parathyroid glands is vitamin D. A number of investigators (7) have shown that a deficiency of this vitamin in chickens leads to a hyperplasia and it has been postulated that this effect represents a compensatory mechanism. It is thought that stimulation of the parathyroid apparatus constitutes an effort on the part of the organism to elevate the Ca-P levels of the blood, lowered by vitamin D deficiency. Evidence of increased function in vitamin-D deficient children is provided by Hamilton and Schwartz (8).

There are others, however, who hold to the theory that vitamin D, rather than the lack of it, stimulates the parathyroid glands. It is pointed out by these workers (9) that the administration of this vitamin helps to alleviate the ill effects of parathyroidectomy. Grant and Gates (10) found that the exposure of rabbits to ultraviolet light consistently caused a hyperplasia of the parathyroid glands, amounting in some instances to more than half the normal weight.

EXPERIMENT. In order to determine the relative effects of renal injury, vitamin D and pregnancy upon the size of the parathyroid glands, 6 groups of rats were used.

The group of normal controls consisted of 9 littermates of the rats which

formed the second, or partially nephrectomized, group. The method of reducing substantially the amount of functioning renal tissue in the latter group was the same as described in a previous experiment (11). The left kidney was removed under ether anesthesia when the animal weighed from 60 to 80 grams. In a second operation, following the first by approximately one week, the upper half of the right kidney was ligated.

A third group of rats was fed an average of 100,000 I.U. of irradiated ergosterol over a period of 27 days prior to autopsy. This was in addition to their balanced basal ration. The same excessive amount of vitamin D was given to paired littermates of this group, the members of which were previously subjected to partial nephrectomy. This fourth group was set up to evaluate the effect of summation of vitamin D and nephrectomy in the same animal.

A fifth group of 10 female rats were made pregnant and autopsied within the last 2 or 3 days of gestation. Ten paired littermates of group 5 were similarly

TABLE 1

Volume of parathyroids, summary of statistical data

GROUPS	NUMBER IN GROUP	MEAN VOLUME $\times 10^6\mu$	STANDARD DEVIATION OF MEAN $\times 10^6\mu$
Untreated control	9	114	10.6
Nephrectomy	9	302	48.6
Vitamin D	7	151	18
Vitamin D with nephrectomy	7	390	92.8
Pregnancy	10	161	12.8
Pregnancy with nephrectomy	10	259	25.8

The usual statistical formulae were used throughout; in obtaining standard deviations, the correction $(n - 2)$ was used for groups of ten members, and $(n - 3)$ for groups with less than ten members.

autopsied during the last days of pregnancy, the onset of which followed partial nephrectomy. Again, this dual procedure was employed to determine the combined effects of the 2 factors, pregnancy and renal injury.

The rats of all 6 groups were killed by exsanguination.[1] The parathyroids with attached thyroid tissue were removed, fixed in Bouin's solution, sectioned serially at 6 μ and stained with hematoxylin and eosin.

The volume of the parathyroids was determined by the method described by Meredith (12), whereby interval sections from the glands were projected at a uniform magnification. The areas of projection were determined by means of planimeter and curves drawn for each organ using the serial position of the section as measured in micra as the abscissa and the area of projection of that section as the ordinate. A smooth curve was drawn connecting these points and the area under the curve, measured by means of planimeter. From this figure, an index of the total volume of parathyroid tissue was easily derived.

RESULTS. A summary of the statistical data (table 1) reveals the largest standard deviations within those groups of rats which were subjected either to

[1] Variations in weight and age at autopsy were kept within a very narrow range.

nephrectomy alone, or to nephrectomy in conjunction with some other treatment. This increased intra-group variation is probably related to an accidental difference in the quantitative reduction of functional renal substance. Pappenheimer and Wilens (13) in their studies of 21 cases of chronic renal disease found an increase in the weight of the parathyroids roughly proportional to the severity and extent of the renal lesions and to the degree of clinical insufficiency. In this experiment, ligation of the second kidney was designed to produce comparable increased reductions of renal substance. That this procedure could not obtain identical degrees of damage in a series of animals was expected.

In lines a, b and c of table 2 a comparison is made between groups which differ only in respect to nephrectomy. In each instance the volume of the parathyroids is greater in the nephrectomized group. This excess amounts to 165 per cent in the series not otherwise treated, 158 per cent in the vitamin D treated

TABLE 2

Comparisons between groups

COMPARISON	DIFFERENCE $\times 10^6\mu$	S.D OF DIFFERENCE $\times 10^6\mu$	CRITICAL RATIO
a. Nephrectomy minus control	188	49.7	3.78
b. Vit. D & nephr. minus vit. D	239	94.5	2.53
c. Preg. & nephr. minus preg.	98	28.8	3.40
d. Preg. minus control	47	16.6	2.83
e. Vit. D minus control	37	20.9	1.77
f. Vit. D & nephr. minus nephr	88	104.6	0.84
g. Nephr. minus preg. & nephr.	43	55	0.78

rats, and 61 per cent in the pregnant animals. These differences have a critical ratio over three in the untreated and pregnant series, and are therefore statistically highly significant. The critical ratio of 2.53 in the vitamin D treated series indicates that the difference is fairly significant.

The difference between the untreated control group, and the other groups subjected to nephrectomy, pregnancy or hypervitaminosis-D above is brought out in lines a, d and e. Line a has already been discussed. Differences between animals which were not littermates but which were of comparable age and weight at autopsy are presented in lines d and e.

The volume of the parathyroids in the pregnant group exceeds that in the control group by 40.5 per cent. This difference is fairly significant. The glandular volume of the hypervitaminotic group exceeds that of the control group by 31.5 per cent but the difference is not very significant since the critical ratio falls below 2.0.

Tabulation of the difference among the nephrectomized groups in lines f and g reveals that the nephrectomized group lies midway between the pregnant-nephrectomized group and the vitamin D-nephrectomized group, but does not differ from either to a statistically reliable degree. The smallest volume is

found in the pregnant-nephrectomized rats which gives a value 14.2 per cent less than that for the nephrectomized animals. The nephrectomized group is exceeded by 29.3 per cent by the vitamin D-nephrectomized group.

As it is reasonable to assume that glandular and body weight are correlated, the tendency for nephrectomized rats to weigh 20 to 30 grams less than their non-nephrectomized littermates might serve to decrease slightly the volume of the parathyroids in the former. This would, if anything, further enhance the significance of the tabulated results.

SUMMARY AND CONCLUSIONS

The significance of the three factors to which hyperplasia of the parathyroid glands is ascribed in the literature was tested experimentally in 52 rats, divided into 6 groups. The groups were subjected to no treatment, partial nephrectomy, hypervitaminosis-D, pregnancy, nephrectomy and hypervitaminosis-D, and nephrectomy and pregnancy, respectively.

Partial nephrectomy was associated with a statistically reliable increase in parathyroid volume in rats not otherwise treated as well as in groups which were also made hypervitaminotic or pregnant.

Pregnancy alone was associated with a fairly significant increase in parathyroid volume.

Hypervitaminosis-D alone in rats was associated with a statistically unreliable increase in parathyroid volume.

Within the nephrectomized groups, there was no significant difference between the group not otherwise treated and either that given vitamin D or made pregnant.

A summation effect of two factors, renal damage and pregnancy, each capable of producing hyperplasia of the parathyroids, was not apparent.

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INFLUENCE OF BASAL FOREBRAIN AREAS ON THE ELECTROCORTICOGRAM

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Previous studies have shown that the electrical activity of the cortex may be modified by stimulation of the thalamus and some parts of the basal ganglia. Although the suggestion (Grinker and Serota, 1938) has been made that hypothalamic activity may also affect the cortex, incidental observations during the course of experiments directed primarily at the thalamus (Morison, Dempsey and Morison, 1941; and Morison and Dempsey, 1942) failed to demonstrate such a relation.

In the latter studies, exploration was carried out with single shock or relatively low frequency stimulation, anesthesia was relatively deep, and the effect on cortical areas presumed to have most intimate relation to the hypothalamus, notably gg. cinguli and proreus, was not investigated.

The present study was therefore carried out with the appropriate modification of the above details in the hope of establishing a more precise relationship between hypothalamus and the electrocorticogram. This objective was not achieved, but incidental information of some interest was collected.

METHODS. Cats anesthetized with nembutal were used. Ordinarily the level of anesthesia was such as to just abolish spontaneous movement, small additional amounts being added intravenously as necessary.

Records were taken from various cortical regions by means of small silver bipolar electrodes connected through 5 push-pull stage, condenser-coupled amplifiers to a multi-channel Grass ink-writer. Ordinarily the electrodes were placed upon the arm sensory area, the auditory area, the middle suprasylvian gyrus and in eight experiments the medial surfaces of gg. proreus and cinguli, appropriate removals of the opposite hemisphere being made to allow access to the latter two regions. In other experiments contralateral cortical regions were studied.

Arrangements for orienting the exploring electrodes and supplying stimulating pulses to them were similar to those used in preceding studies (Morison and Dempsey, 1942). At autopsy the brain was fixed in formalin for later identification of stimulating points by gross or frozen microscopic section.

RESULTS. 1. *Responses to single shocks.* The results of single shock activation of the hypothalamus may be dismissed in a word. They were essentially negative. Occasional secondary responses (Forbes and B. R. Morison, 1939) were encountered from the dorsal hypothalamus as described by Morison, Dempsey and Morison (1941), but the anesthesia was not ordinarily deep enough to favor their appearance routinely. The exposure of the gyri cinguli and proreus carried out in these experiments allowed these regions to be added to the now complete list of neo-cortical areas which exhibit this phenomenon.

The primary response recorded from proreus in a small number of cases by Morison and Dempsey (1942) was found routinely in these experiments when stimulating electrodes were in the anterior nuclear mass or anterior thalamic peduncle.

Effects on g. proreus from stimulation of mammillary bodies or the bundle of Vicq d'Azyr or on cinguli via the more complex path suggested by Le Gros Clark (1938) were not encountered.

2. *Responses to repetitive stimulation.* No effects which could unequivocally be attributed to stimulation of hypothalamic nuclei were seen. Two effects, one

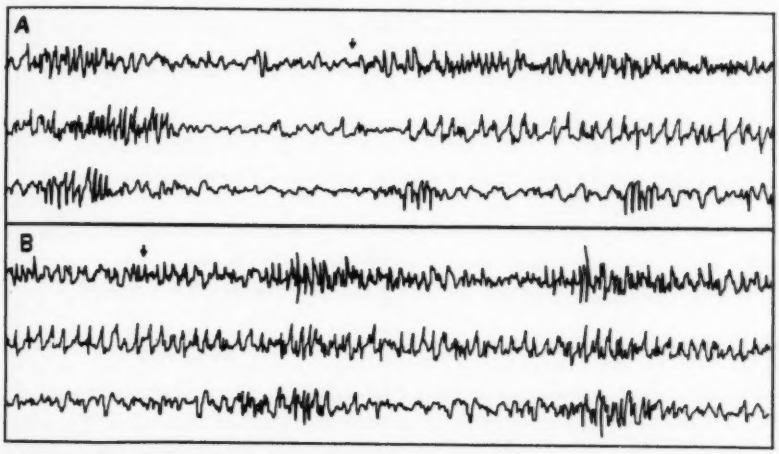

Fig. 1. Suppression of burst activity with increase of "projection" activity on anterior sigmoid gyrus, and 3 per sec. activity on anterior marginal. Note slow disappearance of effect after cessation of stimulation.

Upper tracing: anterior sigmoid gyrus; middle: anterior part of marginal gyrus; lower: middle suprasylvian. Arrows indicate the beginning and end of stimulation of the anterior part of the thalamic fasciculus of Forel, at 60 per second. Twenty-five seconds removed between A and B.

This and the succeeding records were taken at a paper speed of 15 mm. per second and reduced one half.

a generalized inhibition of the intermittent 6–10 cycle bursts normally characteristic of the nembutalized cat (figs. 1, 2 and 3), and the other a slow building up of 2–3 per sec. activity (figs. 2 and 4) were produced when the stimulating electrodes were in the hypothalamus, especially its more dorsal parts, but the excitable region extended laterally through the subthalamus, substantia nigra, etc., and at rostral levels even into the region of the amygdala and pallidum. As has already been shown (Dempsey and Morison, 1943), inhibition of the bursts, in nowise different from that under discussion, may also be obtained from the thalamus and was routinely present in these experiments. Equally good and in several instances more intense effects were encountered ventral to the thalamus in regions which failed to yield other responses typical of thalamic stimulation, but the wide

distribution laterally and rostro-caudally, i.e., from in front of the anterior to behind the posterior commissures, makes it unlikely that either the inhibition or the build up of slow activity belongs specifically to either thalamus or hypothalamus.

Distribution of points giving rise to the build up of slow wave activity was similar to that giving inhibition except that the thalamus was not importantly represented. The points therefore have a distribution similar to, though somewhat wider than, that found by Morison, Dempsey and Morison (1941) for those involved in the "secondary response."

Inhibition was best obtained at stimulus frequencies between 60 and 120 per second, but in favorable instances has been seen at frequencies as low as 15 and

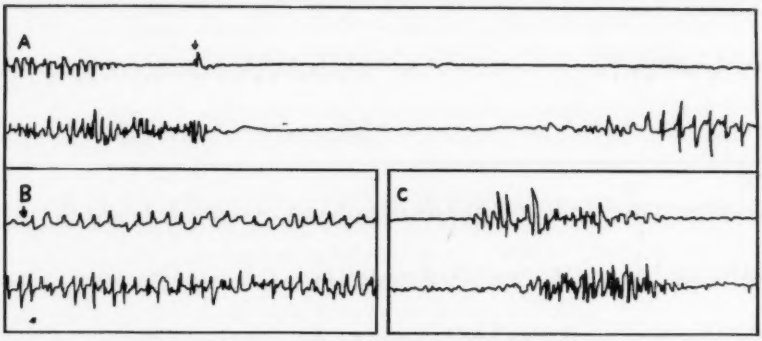

Fig. 2. Complete suppression of burst activity with gradual build up of 3 per second activity on anterior suprasylvian gyrus (lower tracing) and similar though slighter activity appearing as a rebound on the anterior sigmoid (upper tracing). Six seconds removed between A and B. The abnormal activity gradually declined over the succeeding 25 seconds until C the first completely normal burst on an essentially flat background appeared.

Stimulation at 60 per sec. in the region of fields of Forel at the level of the mammillary bodies.

as high as 600 per sec. For complete abolition of the bursts intensities about 2 to 4 times those necessary for producing the thalamic primary response (Dempsey and Morison, 1943) were usually required. In routine exploration therefore a frequency of 60 per sec. and an intensity approximating three times that of the threshold for the primary were adopted. The fact that autonomic responses such as pupillo-dilatation, cardiac acceleration, and respiratory changes were usually nowhere near maximal when the stimulus was applied to the hypothalamus justifies to some extent at least the employment of an intensity which may have contributed to the wide distribution of active points encountered.

Under these conditions, inhibition appeared as an increase in the normal interval between bursts and a reduction in their duration, most intense results being a complete abolition of this type of activity for stimulating periods of 1–3 minutes. In somewhat over half the cases the bursts returned either immediately or within a few seconds after cessation of stimulation (figs. 1 and 3).

In others a longer delay (up to 90 sec.) ensued. The occurrence of such "after discharge" of inhibition could not be correlated with the location of the stimulation point, or the level of anesthesia and only very generally with the intensity, frequency, or duration of stimulation. Not all cortical areas were equally depressed. In general somatic sensorimotor cortex gave the most striking results, the middle suprasylvian gyrus the least (fig. 1). In a few instances in which activity of both hemispheres was recorded, slight inhibition was found on the side opposite to the location of the stimulating electrodes, but this was never at all comparable to the homolateral effects.

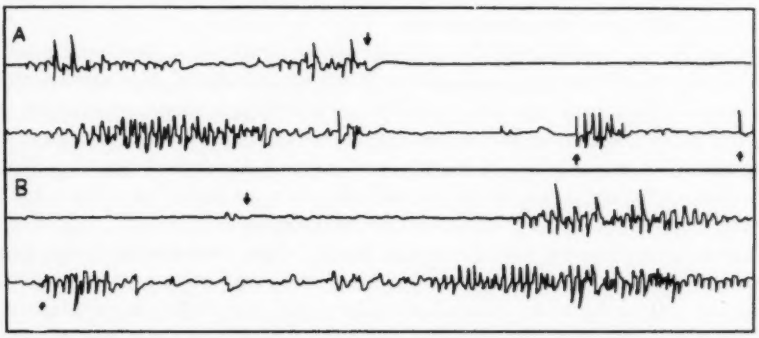

Fig. 3. Complete suppression of burst activity on anterior sigmoid (upper tracing) and superior sylvian (lower tracing) gyri with lack of effect on response to handclap in the latter. Downward arrows indicate beginning and end of stimulation just dorsal to mammillary bodies; upward arrows—handclaps. Ten seconds removed between A and B.

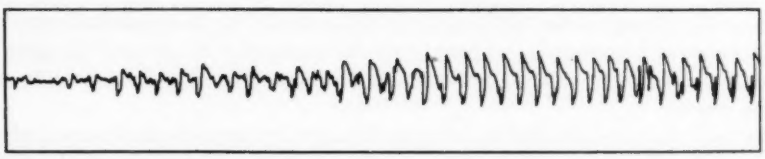

Fig. 4. Gradual build up of spike and wave formation in posterior sigmoid gyrus from stimulation in region of field H_2 of Forel at the level of the anterior border of the mammillary bodies. Stimulation commenced 2 sec. before beginning of record.

If the existing spontaneous cortical activity consisted of intermittent bursts separated by periods of little or no activity, suppression of the bursts resulted in a virtually flat record (figs. 2 and 3) which might later give way to a build up of 3 per second activity (fig. 2, lower tracing). Continuous spontaneous activity of the "projection" type (Dempsey and Morison, 1943) was, however, not suppressed and might even be increased both during stimulation and for some time afterwards (fig. 1, upper tracing). The somewhat random spike-like activity, similar to the secondary responses and attributable to a non-thalamic system (Morison, Dempsey and Morison, 1941), and more continuous slow-wave activity (2-3 per second), either "spontaneously" present in some experiments or

induced by stimulation of the areas described above, were likewise unaffected. Indeed stimulation of subthalamic areas often resulted in prolonged inhibition of bursts with a gradual superimposition of the 3 per sec. activity (figs. 2 and 4).

Responses produced by stimulation of a peripheral nerve (cf. Dempsey and Morison, 1943) or by a sharp handclap (fig. 4) were readily recorded in the presence of prolonged inhibition of bursts.

The building up of slow-wave activity referred to above was not so routinely encountered as the inhibitory phenomena. Very rarely such episodes have occurred "spontaneously" under the experimental conditions used here, and then only during the later stages of an experiment after the possibilities of non-specific damage to the cortex may have arisen.

In slightly over one-half of the present experimental series, prolonged stimulation in the regions already mentioned gave rise to episodes of slow-wave activity. The ease of elicitation was very variable even within a single experiment, and long rest periods were necessary in order to ensure repetition of the episodes in anything like their original form in the course of a series of identical stimuli. Not all cortical areas participated simultaneously in every episode, but at one time or another every area studied exhibited the phenomena.

Various patterns were found varying from a slight increase in rather nondescript and irregular slow waves to the definite wave and spike pattern evolved in the latter part of figure 4. The abnormalities rarely ceased coincidentally with the stimulus, but gradually disappeared over a period of from one to several minutes (fig. 2). Occasionally, after several inductions of the effect, normal activity failed to reappear at all and the experiment had to be discontinued.

Some of the most striking instances of the building up of cortical activity of low frequency were accompanied by increased respiration, and the question arose whether the change in the corticogram might be secondary to an alkalosis or other disturbance of homeostasis. Forced artificial respiration to an extent far greater than that consequent on stimulation failed, however, to induce significant changes in the record.

Perhaps the most conclusive evidence that the cortical changes were a direct result of the stimulus was derived from experiments in which the cortical activity was registered from both hemispheres. In such cases the abnormal waves usually appeared only on the stimulated side. In occasional instances bilateral effects were encountered, but these were always less marked on the side opposite to that stimulated.

Discussion. The most plausible deduction that may be made from the distribution of points giving rise to inhibition of the cortical bursts is that this activity resides in fiber systems passing through the dorsal hypothalamus and subthalamus and regions immediately rostral and caudal thereto and represent lemniscal, lenticular, and possibly conjunctival systems on their way to the intralaminar nuclei concerned with the production of cortical bursts (Morison and Dempsey, 1942; Morison, Finley and Lothrop, 1943). Since stimulation of peripheral nerve (Dempsey, Morison and Morison, 1941), or the striatum (Dus-

ser de Barenne and McCulloch, 1938), have both been reported as inhibitory for at least some elements of the electrocorticogram such an interpretation is plausible enough. In our hands, at least, stimulation just ventral to the thalamus was ordinarily productive of more striking effects than was activation nearer to the origin of the fiber systems (for instance, the pallidum). This, however, may be explained by the greater concentration of fibers as they approach their endings in the thalamus.

It is important to emphasize that the inhibition affects the cortical bursts specifically and leaves the discrete responses evoked by sensory stimulation, the 2–3 per sec. activity induced by basilar stimulation, and two forms of spontaneous activity completely unmodified (p. 414). Not only does this suggest that the inhibition takes place in the thalamus and does not represent a generalized depression of the cortex, but it supports the general thesis that the electrocorticogram is a complex resultant of activity in relatively discrete systems separable morphologically and functionally from one another.

Little can be said about mechanisms underlying the production of the build-up activity. Apparently the thalamus is not importantly involved since the most active point was invariably ventral to it.

Because of the fact that these experiments failed to uncover any influence on the electrocorticogram when the stimulating electrodes were deep in the hypothalamus proper which was not also produced from more dorsal areas which extended both laterally and rostrocaudally, it was impossible to confirm Grinker and Serota in their view that the hypothalamus is importantly concerned with the electrical activity of the cortex. Ablation of the hypothalamus (Dempsey and Morison, unpublished), unless so extensive as to involve the blood supply to the thalamus, has also failed to suggest such a relation. The sort of change illustrated by Grinker and Serota as supporting their contention is more easily explained as a result of cutting the lemniscus (Cf. Dempsey and Morison, 1943).

SUMMARY

1. Single shock stimulation of the hypothalamus failed to produce significant changes in the electrical activity of any major cortical area.

2. Repetitive stimulation (15–600 per sec.) produced two significant effects: *a*, a generalized depression of the intermittent bursts of 5–10 per second frequency unaccompanied by suppression of various other types of cortical activity (figs. 1, 2, 3); *b*, a gradual and prolonged building up of slow-wave activity (2–3 per sec.).

3. Active points were found not only in the hypothalamus but in a relatively wide area lateral and posterior to it. Effect *a* but not *b* was also produced from the thalamus especially its medial part.

4. It appears that the inhibitory effect may be attributed to the activation of various fiber systems running through the dorsal hypothalamus to reach the areas of the thalamus shown by other studies to be important in controlling the intermittent cortical bursts.

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STUDIES ON THE LINGUO-MAXILLARY REFLEX

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Gellhorn, Cortell and Carlson showed that the excitability of the autonomic centers in hypothalamus and medulla oblongata was greatly increased in anoxia whereas the reactivity of the somatic centers which was tested by stimulation of the same area was diminished. The experiments seemed to indicate a fundamental difference in the excitability of somatic and autonomic centers. The present experiments were designed to contribute further material to this problem and to include also conditions other than anoxia. They involve in contradistinction to our earlier work not direct but reflex stimulation of autonomic and somatic centers. Since the main vegetative centers are located in the medulla oblongata and adjacent parts of the pons (cf. Lim and collaborators for the sympathetic myelencephalic center, and Pitts, Magoun and Ranson for the respiratory center) it was decided to study the effect of changes in internal environment on respiration, blood pressure and a somatic reflex, the linguo-maxillary reflex (1, 2, 3, 5) whose center is located in the same section of the central nervous system. The action of anoxia, asphyxia, hypercapnia and hypoglycemia was investigated.

METHOD. The results are based on experiments performed on 48 cats anesthetized with 100 mgm./kgm. chloralose subcutaneously or 40 mgm./kgm. pentothal. Some additional experiments were performed on dogs with pentothal-chloralose anesthesia. Preparation of the digastric muscle and recording of its contraction was carried out as described by Blier and Kleitman. The blood pressure (Hürthle or Hg-manometer) and respiration were recorded in the usual manner. The gas mixtures prepared by means of flow meters were inhaled from Douglas bags. Condenser discharges at the rate of one per second were applied to the tongue in order to elicit the linguo-maxillary reflex. The blood sugar was determined by the Somogyi modification of the Shaffer-Hartman method.

RESULTS. 1. *Hypercapnia.* The fundamental difference in the reactivity of somatic and autonomic centers at the medullary levels is well illustrated by the study of the effects of 7.4 per cent carbon dioxide on blood pressure, respiration, and linguo-maxillary reflex. Whereas respiration increases and the blood pressure rises the linguo-maxillary reflex decreases. The effects were reversible and occurred uniformly in 23 animals.

The elimination of the chemo-receptors by bilateral vagotomy and denervation of the carotid sinus area did not change the type of reaction which is illustrated in table 2.

Hyperventilation during which the respiratory minute volume was increased

TABLE 1

Effect of restoring normal blood sugar levels upon the height of contraction of the linguo-maxillary reflex in insulin hypoglycemia cats

NO.	BLOOD SUGAR LEVEL BEFORE GLUCOSE INJECTION	AMOUNT OF GLUCOSE INJECTION	MAXIMAL CHANGE IN RESPONSE IN PER CENT OF CONTROL LEVEL	TIME AFTER INJECTION TO MAXIMAL RESPONSE	BLOOD SUGAR LEVEL AFTER INJECTION
	mgm. per cent	*cc. 7 per cent*	*per cent*	*min.*	*mgm. per cent*
1	48.3	15	+50	4	206
2	36.5	7	+300	9	93.4
3	38.2	25	+350	2	142
4	25.8	10	+250	4	129
5	34.4	10	+250	3	103
6	40.8	10	+400	2	93.5
7	59.1	15	+250	2	221
8	37.6	10	+50	3	137
9	47.3	15	+100	3	144
10	19.3	15	+400	3	108
11	16.4	20	+400	5	184
	Effect of elevating blood sugar above normal levels				
1	104	15	No change		183
2	166	30	No change		236

TABLE 2

The effect of 7.4 per cent CO_2 on the linguo-maxillary reflex

A. Blood sugar normal (no insulin)

NO.	DURATION OF PERIOD OF INHALATION OF 7.4 PER CENT CO_2	MAXIMAL EFFECT IN PER CENT OF CONTROL	PERIOD OF RECOVERY TO 75 PER CENT CONTROL LEVEL AFTER REMOVAL OF CO_2
	min.	*per cent*	*min.*
1	10	−75	3
2	8	−100	3½
3	8	−90	2
4	14	−95	4
5	6	−100	3
6	10	−50	3
7	3	−90	1
8	6	−60	3
9	10	−50	2

B. Blood sugar reduced by insulin

Effect of inhalation of 7.4 per cent CO_2 upon the height of contraction of the linguo-maxillary reflex in the insulin hypoglycemia animal

NO.	DURATION OF CO_2 INHALATION	MAXIMAL CHANGE IN RESPONSE IN PER CENT OF CONTROL LEVEL	PERIOD OF RECOVERY TO 75 PER CENT CONTROL LEVEL	BLOOD SUGAR LEVEL
	min.	*per cent*	*min.*	*mgm. per cent*
1	1½	−100	5	36.5
2	2½	−75	4	63.4
3	2	−100	3	25.8
4	1½	−100	8	25.8
5	1½	−100	7	34.4
6	1½	−75	2½	40.8
7	3	−50	2	59.1
8	1½	−75	2	36.7

approximately 8 times led in 12 experiments on 3 animals to an increase of the linguo-maxillary reflex of 300 to 400 per cent. Here again a marked contrast existed between vegetative and somatic centers in as much as the excitability

of the autonomic centers decreased as indicated by the apnea following the period of hyperventilation and by a slight fall in blood pressure.

2. *Anoxia and asphyxia.* In 20 experiments in which 6.2 per cent oxygen was inhaled it was found in all but 3 that this degree of anoxia resulted either in a complete disappearance of the linguo-maxillary reflex or in considerable reductions. The effects were reversible in almost all instances within 10 minutes after readmission of air. In 3 instances this oxygen-nitrogen mixture failed to cause a change in the linguo-maxillary reflex. In all experiments an increased respiratory response and a rise in blood pressure accompanied this decrease of the linguo-maxillary reflex. Figure 1 illustrates a typical effect.

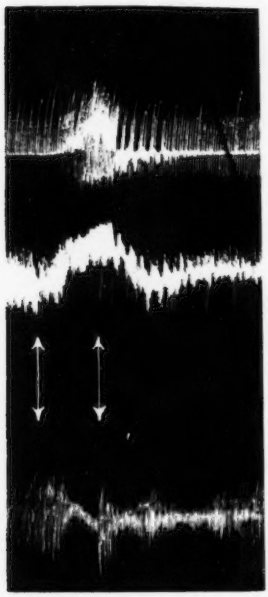

Fig. 1. Effect of 4.5 per cent O_2 for 2 min. on the linguo-maxillary reflex of the dog anesthetized with Na-amytal 40 mgm./kgm. intraperitoneally and chloralose 30 mgm./kgm. intravenously. Recordings from above downward: Respiration, blood pressure, linguo-maxillary reflex.

This experiment was repeated in 6 cats in which the carotid sinus area had been denervated and the vagi cut in order to determine the rôle of the chemoreceptors. It was found that under these conditions anoxia likewise either greatly reduces or completely abolishes the linguo-maxillary reflex. However, it was observed that the period of reduced reflex response was preceded by a temporary phase during which the reflex was increased. Since it is known from the investigations of Gellhorn and Lambert that elimination of the chemoreceptors leads to a fall in blood pressure in response to anoxia instead of the rise seen in normal animals it was thought that possibly the reduction in blood flow associated with a fall in blood pressure may account for the appearance of a temporary increase in the linguo-maxillary reflex response. It was found indeed that this reaction of the reflex could be reproduced in normal cats by administering 6.2 per cent oxygen while both carotid arteries were clamped.

Asphyxia induced by clamping of the trachea caused in 4 experiments a complete or nearly complete disappearance of the linguo-maxillary reflex while respiratory efforts were greatly increased and the blood pressure rose.

3. *Hypoglycemia.* Twelve experiments were performed upon six cats. The animals were rendered hypoglycemic by the intramuscular injection of from 4 to 8 units of insulin per kilogram one to two hours before the beginning of the experiment. A venous blood sample was obtained for sugar analysis. From 7 cc. to 25 cc. of 7 per cent glucose was then injected intravenously. Within from 2 to 9 minutes after the injection the height of contraction of the reflex increased from 50 per cent to 400 per cent above that recorded at the hypoglycemia level. A second venous blood sample was then taken for sugar analysis. Additional insulin was later injected and the experiment repeated. The results illustrated in table 1 showed that the injection of glucose restored the linguo-maxillary reflex although an increase of blood sugar above the normal value was without effect on the reflex response, as control experiments indicated.

These observations illustrate again the fundamental difference in the reactivity of somatic and vegetative centers in the same part of the central nervous system in as much as hypoglycemia is accompanied by an increased respiratory activity leading to a diminution in the carbon dioxide content of the arterial blood and a decrease in the carbon dioxide tension of the alveolar air (Domm and Gellhorn). Earlier studies of Gellhorn and collaborators (1938–1940) likewise demonstrated an increased reactivity of the vasomotor center in hypoglycemia.

Table 2 shows the effect of carbon dioxide on the linguo-maxillary reflex at normal and reduced blood sugar levels. It is evident that when the blood sugar had been reduced by insulin, carbon dioxide becomes more effective and causes a more rapid decline of the linguo-maxillary reflex than is observed at a normal blood sugar level. Moreover, the period of recovery is somewhat lengthened in the hypoglycemic experiments as compared to those performed without the use of insulin. These experiments are of interest in view of the fact that Gellhorn, Kiely and Hamilton observed that the pressor response elicited by carbon dioxide is inversely related to the blood sugar level. Apparently the effects of carbon dioxide are potentiated under condition of hypoglycemia. Whereas this potentiation results in a greatly increased blood pressure response to carbon dioxide, it causes a marked acceleration of the depressant action which carbon dioxide exerts on a somatic medullary reflex.

Discussion. The experiments reported in this paper give clear evidence for the fact that alterations of the internal environment with respect to carbon dioxide, oxygen tension and blood sugar act in opposite manners on somatic and vegetative centers of the medulla. Whereas anoxia and hypercapnia cause a rise in blood pressure and an increased respiratory minute volume the linguo-maxillary reflex whose center is located in the brain stem is greatly depressed. Hypoglycemia which stimulates respiration and increases the reactivity of the vasomotor center to reflex-stimulation as well as to direct stimulation depresses

the linguo-maxillary reflex. When two of the factors such as carbon dioxide and low blood sugar are combined greatly augmented effects are obtained on both somatic and vegetative centers. Under these conditions the fundamental difference in the reactivity of autonomic[1] and somatic centers is retained.

The physiological significance of this fundamental difference is obvious. The increased respiratory activity under conditions of hypercapnia and anoxia tends to restore the internal environment and to minimize the effects which these disturbances in homeostasis would otherwise have on the somatic nervous system. This interpretation is applicable to the conditions of hypoglycemia because the increased excitability of the sympathetic myelencephalic center tends to restore the blood sugar through sympathetic-adrenalin discharges. Moreover, the increased respiration tends to raise the oxygen tension in the blood which is of importance since the effect of hypoglycemia on the brain can be eliminated by the inhalation of pure oxygen (Kessler and Gellhorn).[2]

SUMMARY

Somatic and autonomic reactions whose reflex center is located in the brain stem were studied under conditions of disturbances of the internal environment. It was found that hypercapnia while exciting respiration and blood pressure decreases the linguo-maxillary reflex. Hyperventilation resulting in a decreased excitability of the respiratory and vasomotor centers caused a marked increase in the intensity of the linguo-maxillary reflex. The fundamental difference in the reactivity of somatic and autonomic brain stem centers is further illustrated by experiments on anoxia and hypoglycemia. These conditions depress the linguo-maxillary reflex while they excite the autonomic medullary centers. If carbon dioxide is inhaled at various blood sugar levels it is found that the depressant action of carbon dioxide on the linguo-maxillary reflex is inversely related to the blood sugar level whereas its excitatory effect on the vasomotor centers increases with falling blood sugar. The physiological significance of these reactions for the protection of the brain and the maintenance of homeostasis is emphasized.

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[1] The respiratory center innervating striated muscles is ordinarily not considered a part of the autonomic nervous system. However, it is likewise distinguished from the somatic nervous system by the persistence of its activity when voluntary control is eliminated. The present investigation showing that respiratory and vasomotor centers react similarly and in contrast to closely located somatic centers, suggests the classification of the respiratory center as a part of the autonomic system.

[2] For further discussion cf. Gellhorn, 1943.

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OBSERVATIONS ON THE EFFECT OF CERTAIN DRUGS ON THE SMALL BLOOD VESSELS OF THE RABBIT EAR BEFORE AND AFTER DENERVATION[1]

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The exact nature of the rôle of sympathetic innervation in the reactions of blood vessels has long been a subject of interest to physiologists, neurologists and clinicians (1–7). Much work has been done on this question and many important observations have been made. There are still certain aspects of the problem, however, which remain either vague or controversial. Most of the studies made previously have been carried out with the experimental animal under general anesthesia and, in many instances, changes of the caliber of the blood vessels have been inferred by measurements of temperature, blood pressure or alterations of blood flow. The objects of the present study were 1, to observe directly the reactions of the small blood vessels of the ear of the rabbit to certain drugs (epinephrine hydrochloride, ephedrine, ergotoxine and pitressin); 2, to observe the reactions to these drugs of the minute vessels in the innervated and in the denervated ear; 3, to determine the rôle played by the auricular nerves in motor responses of the minute blood vessels of the ear by direct observations of the reactions of these blood vessels to stimulation of the nerves; 4, to correlate the reactions of the blood vessels with evidences at necropsy of regeneration of nerves, and 5, by these means to determine the function performed by the nerves which supply these blood vessels. For this study we have found a modification of the Clark transparent chamber inserted into the ear of the rabbit satisfactory.

METHOD. The transparent chamber we have used is similar to that which has been described by Ebert, Florey and Pullinger (8). This type of window is fashioned on a lathe from a bar of lucite. The advantages over the original one used by Sandison (9) are 1, that the new type does not warp; 2, that it is more transparent than the original type, and 3, that it may be used repeatedly if desired.

Healthy albino rabbits, weighing between 2 and 4 kgm., were used for this study. All surgical procedures, but not the subsequent observations, were carried out with the rabbit under anesthesia produced by pentobarbital sodium (25 to 35 mgm. per kilogram of body weight). The window was inserted at the junction of the middle and distal thirds of the ear between the marginal vein and the central vessels. Space does not permit a detailed description of the operative procedure for insertion of the window.

Most of these windows were fully vascularized at the end of four weeks. Ob-

[1] Abridgment of thesis submitted by Doctor Levinson to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Medicine.

servations on the reaction of the blood vessels to drugs were made from four to six weeks after the insertion of the window. Particular attention was given to the response of the arterioles to injections of epinephrine, ephedrine, pitressin and ergotoxine. Constriction of the arterioles was graded on the basis of 1 to 4; 1 representing slight constriction; 2, moderate constriction (lumen diminished 50 per cent); 3, fairly marked constriction (still slight evidence of a lumen remaining); 4, marked constriction (complete disappearance of the arteriolar lumen). The temperature of the room registered from 21 to 26.5°C. during these studies. Injections of the drugs were made into the marginal vein of the ear opposite the one being observed. If there was constriction of the arteriole on insertion of the needle, we waited until the lumen had returned to its original caliber before starting injection of the drug. Control injections of isotonic saline solution did not produce any response in the vessels. Observations were carried out by means of a compound microscope at a magnification of 200. No attempt was made to measure the caliber of the vessels, since slight movement of the rabbit and the evanescence of some of the reactions made accurate measurement of the vessels difficult. In addition to direct observation, photographs were made of typical responses of the minute vessels to the various drugs. The observations were made on a series of nine animals. Repeated observations were made on each animal with each drug. The most consistent reaction produced by repeated doses of each drug was determined for each animal.

The epinephrine used was a stock solution of 1:1,000. Further dilutions were made with physiologic salt solution. The smallest dose of epinephrine to which any animal reacted was 0.05 cc. of 1:1,000,000 solution. With this dose as a starting point, the dose was increased by increments of 0.05 cc. until a grade 4 response was obtained. It was not necessary to consider the weight of the rabbit since the same doses of epinephrine were used for each animal before and after denervation.

To test the effect of ephedrine, crystals of ephedrine (Burroughs, Wellcome and Co.) were dissolved in physiologic saline solution in a strength of 10 mgm. per cubic centimeter. Doses of 1 mgm. per kilogram of body weight were administered at intervals of ten to fifteen minutes until there was a definite diminution of response but the number of successive doses never exceeded four.

Dilutions of pitressin (Parke, Davis & Co.) with physiologic saline solution to a strength of 2.0 pressor units per cubic centimeter were made just prior to injection and given in doses of 0.1 pressor unit per kilogram of body weight; the same procedure was carried out as with ephedrine.

To test the effect of ergotoxine, 5 mgm. of powdered ergotoxine alkaloid (Burroughs, Wellcome and Co.) was dissolved in 0.5 cc. of absolute alcohol, then made up to a volume of 5 cc. with distilled water and given in doses of 0.5 mgm. per rabbit. Administration of the dose of ergotoxine was preceded in each instance by administration of the smallest dose of epinephrine that produced a response of grade 4. Also in each instance administration of the dose of ergotoxine was followed by administration of a similar dose of epinephrine in order to observe any alteration of the response to epinephrine caused by ergotoxine.

After the foregoing studies had been completed, denervation of the ear was done. This consisted in removing pieces 15 to 20 mm. long of the dorsal and great auricular nerves of the ear in which the window had been placed. On the same side the peripheral portion of the cervical sympathetic chain, together with the superior cervical ganglion, was avulsed. The vessels were observed immediately after this operation and at intervals of five, ten, fourteen, twenty-one days and so forth after denervation. Studies of the action of drugs were also carried out at the intervals just mentioned.

Necropsies were performed on the animals and the condition of the auricular and cervical sympathetic nerves was noted.

Similar observations were made on one rabbit on which the entire operative procedure for denervation was carried out, except that the nerves were not cut. This animal served as a control on the effects of the operative procedures.

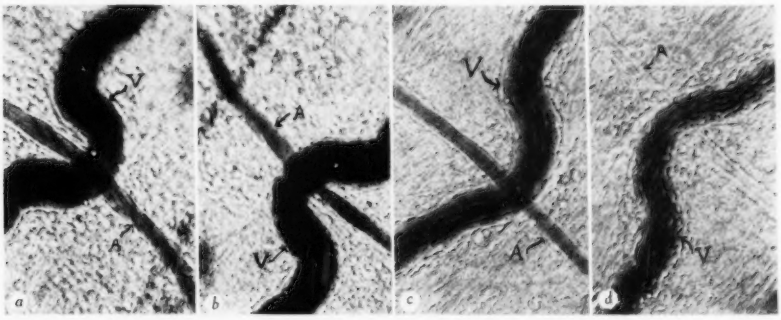

Fig. 1. Effect of denervation on the response of small blood vessels to administration of epinephrine (×200). *a*, appearance of arteriole (A) and venule (V) in the innervated ear; *b*, appearance of same vessels as seen in *a* thirty seconds after 0.2 cc. of 1:1,000,000 solution of epinephrine had been given; *c*, appearance of same vessels as seen in *a* ten days after denervation of the ear; *d*, appearance of same vessels as seen in *c* after 0.2 cc. of 1:1,000,000 solution of epinephrine had been given.

Results. *Epinephrine.* The reaction of the vessels in the window of the innervated ear to epinephrine was usually a constriction of the arterioles within twenty seconds after the injection (fig. 1*a* and *b*). It was observed that not all the arterioles in the window responded equally to the same dose of epinephrine. At times, after administration of small doses of epinephrine, there were noted at irregular intervals along the course of an arteriole constrictions which gave the vessel the appearance of a string of sausages This phenomenon tends to support the smooth muscle motor unit concept of Fulton and Lutz (10). The maximal constriction lasted usually from thirty seconds to one minute. Dilatation was frequently observed for a few seconds after relaxation of the vessel. There was a slight narrowing of the lumina of the venules accompanying the arteriolar constriction. Independent constriction of the capillaries was not observed. So long as any blood was flowing through the arterioles the capillaries remained open. It was only when arteriolar flow was shut off completely that the capillary lumina seemed to disappear.

After denervation (fig. 1*c* and *d*) it was observed in all instances that the does of epinephrine required to produce the grade 4 response was definitely less than for the vessels in the innervated ear. In most instances there was only a slight difference in the size of the necessary dose but in a few cases the difference was very great. This altered response was usually present when the animal was first studied at five days after denervation. It was invariably present at ten days after denervation. In addition the response to epinephrine usually occurred more slowly and lasted longer than in the innervated ear. This increased response to epinephrine disappeared in a few cases after about four weeks following denervation.

Ephedrine. In the animals that had innervated ears ephedrine had the same general effect as epinephrine. However, the effect of ephedrine was more inconsistent than that of epinephrine and usually did not appear until about a minute after injection. The maximal arteriolar constriction usually lasted from three to four minutes. In each case the vessels showed either a diminished or a negative response to repeated doses.

The response of the vessels of the denervated ear to ephedrine was not greatly different from that of the vessels of the innervated ear. In a few animals after denervation of the ear more ephedrine had to be given before the vessels became refractory to the action of the drug than had been necessary when the nerves were intact.

Pitressin. Pitressin had the most powerful vasoconstrictor action on the vessels of the ear of any of the drugs used. Its effect, like that of epinephrine, was chiefly on the arterioles. Independent capillary constriction was not observed. Abell and Page (11) and Barrett (12) have also reported that they did not observe independent capillary constriction after injections of pitressin. The arteriolar constriction usually occurred within twenty to thirty seconds after injection. The maximal effect in most cases lasted from three to ten minutes. As with ephedrine, the effect on the arterioles of repeated doses either became diminished or was negative.

In the denervated ear the response was much the same as in the innervated ear with one exception. In those animals in which there had been diminished response or absence of response of the auricular vessels to repeated doses, the same vascular response occurred, after denervations, with each repeated dose (fig. 2).

Ergotoxine. Almost without exception in animals that had innervated ears ergotoxine produced a definite constriction of the arterioles which usually began about thirty seconds after injection. The maximal constriction lasted from two to fifteen minutes. This was then followed by a marked dilatation of the capillaries and a moderate dilatation of the venules. Temporary arteriolar dilatation also was observed occasionally after the period of constriction. The vessels of the innervated ear after injection of a dose of epinephrine which followed the administration of ergotoxine showed no effect whatever or a definitely diminished effect. In those cases in which there was some constriction of the arterioles when administration of epinephrine followed that of ergotoxine, there was not any effect on the capillaries.

The response of the vessels of the denervated ear to ergotoxine was the same as that of the vessels of the innervated ear with two exceptions. First, ergotoxine produced a definite constriction of the arterioles in those few animals

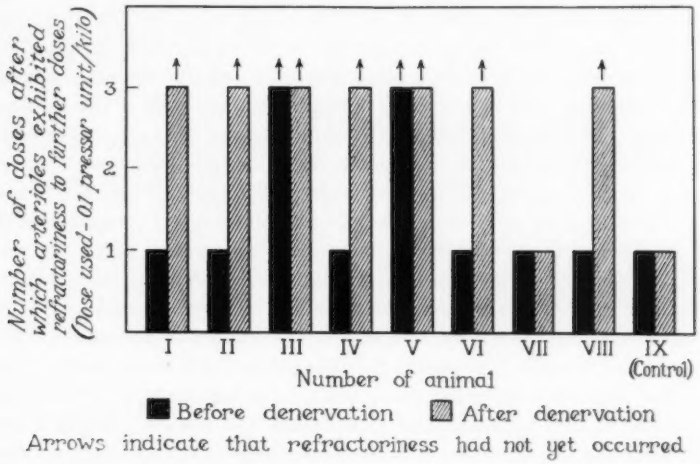

Fig. 2. Number of doses of pitressin required to produce refractoriness of arterioles to further doses. Effect before and after denervation.

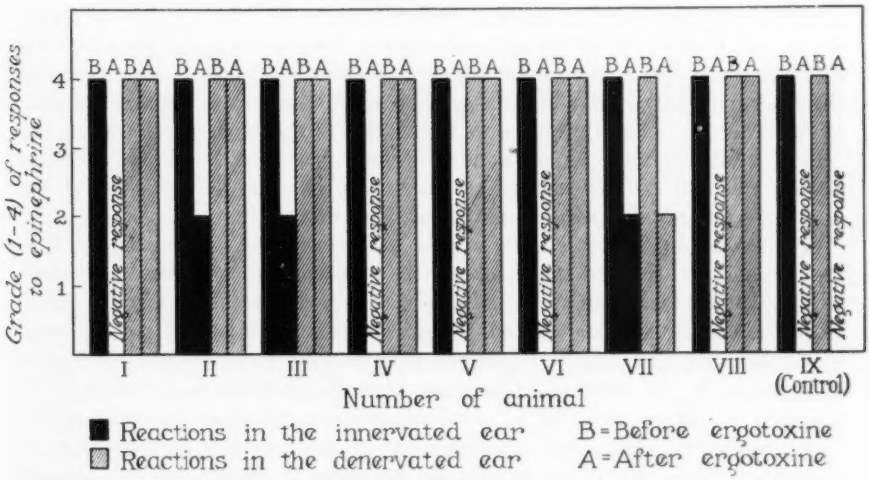

Fig. 3. Effect of denervation on the inhibitory action of ergotoxine

which had not shown any response of the arterioles to ergotoxine before denervation. Second, there was an alteration of the inhibitory action of ergotoxine, occurring usually between the fifth and the fourteenth day after denervation (fig. 3). During this period except in one case ergotoxine apparently had little

or no effect on the action of epinephrine. However, in those animals observed for more than two weeks the effect of ergotoxine became the same as it had been before denervation.

Effects of stimulation of the peripheral ends of the severed nerves. At the end of the first eight days of growth Clark, Clark and Williams (13) demonstrated nerves in the tissue which had grown into the chamber inserted into a rabbit's ear. It has also been shown (20) that stimulation of the peripheral end of the cervical sympathetic chain results in a swelling of the endothelial cells of the capillaries in this chamber. Meltzer and Meltzer (3) found that in the majority of cases, the third cervical nerve carries the vasomotor fibers for the blood vessels of the rabbit's entire ear, except for a comparatively small region around the lower two-thirds of the central artery, which is supplied by the cervical sympathetic nerves. In each of the animals we have studied except the control animal 9, we have recorded the changes of the vessels in the chamber subsequent to stimulation of the peripheral ends of the severed auricular and cervical sympathetic nerves.

Stimulation of the peripheral end of the cut cervical sympathetic chain invariably produced marked constriction of the arterioles in the chamber (table 1). This usually began about twenty seconds after the beginning of stimulation and lasted for one to two minutes after stimulation had ceased. Slight narrowing of the venules was also observed. No definite independent capillary constriction was observed. The disappearance of the capillary lumen seemed to be secondary to marked constriction of the lumen of the supplying arteriole.

Stimulation of the peripheral end of either the dorsal or the great auricular nerve did not produce a consistent response (table 1). In one animal no response was observed on stimulation of either of these nerves. In other animals a response was observed on stimulation of one nerve but not on stimulation of the other. In only one animal was a response obtained by stimulation of each of these nerves. When response to stimulation of these nerves did occur, the great auricular nerve produced a more marked effect than the dorsal auricular nerve. As with the cervical sympathetic chain no active constriction of the capillaries was noted after stimulation of these nerves.

Observations on the status of the vessels after denervation. Immediately after the sectioning of the auricular and cervical sympathetic nerves there appeared to be a definite dilatation of the arterioles, venules and capillaries in the chambers of all of the animals studied. (Animals had been anesthetized for some time when this was done.) When the animals were observed five days after denervation, it was noted in all instances that the arterioles were definitely diminished in caliber, although in some animals the reduction was only slight. The constriction seemed to be independent of environmental temperature or the degree of quietness of the animal and it seemed to persist. In two animals in which the hypersensitivity to epinephrine had disappeared at the end of about four weeks after denervation, there was concomitantly a return of the arterioles to their original caliber.

Postmortem findings on the sectioned nerves. There was not any evidence of

regeneration of the cervical sympathetic chain in any animal in the series. In only one animal was there found gross evidence of regeneration of the auricular nerves. In this rabbit there was an actual bridging of the great auricular nerve at the point where it had been sectioned forty-four days previously. The whole nerve appeared larger than the one on the intact side and there were bulbous enlargements along its course. In this animal there was a nodular swelling

TABLE 1

Effect of stimulation of nerves supplying the ear and condition of severed nerves at necropsy

RABBIT	EFFECT OF STIMULATION OF VARIOUS NERVES ON CONSTRICTION, GRADE, OF VESSELS				GROSS EVIDENCE OF REGENERATION OF NERVE AT NECROPSY	DAYS AFTER OPERATION
	Vessels	Dorsal auricular	Great auricular	Cervical*		
1	Arterioles Capillaries	0 0	4 0	Not observed	None	30
2	Arterioles Capillaries	0 0	2 0	2 0	None	43
3	Arterioles Capillaries	0 0	3 0	4 0	None	37
4	Arterioles Capillaries	2 0	4 0	3 0	Great auricular nerve	44
5	Arterioles Capillaries	2 0	0 0	4 0	Not observed	
6	Arterioles Capillaries	3 0	0 0	4 0	None	10
7	Arterioles Capillaries	0 0	0 0	4 0	None	15
8	Arterioles Capillaries	0 0	4 0	2 0	None	14

* Pupil dilated.

of the dorsal auricular nerve at the proximal end of the distal segment of the cut nerve. The proximal segment could not be found. There was no evidence of regeneration of this nerve.

COMMENT. The increased sensitivity to epinephrine and the return of "vascular tone" after denervation remain enigmas. It seems, however, that these phenomena are associated with the interruption of the sympathetic nerves. It is very interesting that the increased sensitivity to epinephrine and the diminished caliber of the vessels in a few animals disappeared when according to the work of others (14, 15) it might be suspected that regeneration of nerves

had taken place. However, we were able to demonstrate regeneration anatomically in only one animal, although there was evidence of return of function in the others.

We have no adequate answer as to why the blood vessels exhibited increased sensitivity to drugs after interruption of their sympathetic nerve supply. Rosenblueth and Morison (16) suggested that denervation increases the permeability of smooth muscle cells to epinephrine. The slowness of the response and the increased duration of the effect of epinephrine after denervation would tend to indicate some alteration of a hypothetical receptive substance rather than a change of permeability. Ascroft (5) expressed the opinion that there is not any reason to doubt that sensitization developing after denervation is due to change of the peripheral structures. Grant (2) suggested that there must be some as yet unidentified pressor substance in the circulation which would account for the return of "vascular tone." This still would necessarily imply an altered reactivity of the blood vessel to this pressor substance.

The altered effect of ergotoxine after denervation lends support to the idea of a change of some mechanism associated with the blood vessel. Cannon and Rosenblueth (17) have indicated that the blocking effect of ergotoxine occurs between the "receptive substance" and the "specific response." If some alteration of this receptive substance had occurred after denervation, making the substance more responsive to epinephrine, would it not then be more difficult for a given dose of ergotoxine to inhibit this response after denervation? This is apparently just what occurred.

The fact that these reactions have been the same, on the whole, as those demonstrated by others (9, 11, 18–20) using different methods justifies the use of the tissue grown into the chamber inserted into the rabbit's ear as a physiologic experimental unit. The fact that the surgical procedures necessary to prepare the rabbits for our observations—procedures which were performed with the animals under anesthesia—preceded the observations by several weeks and that therefore the observations have been carried out successfully in the absence of anesthesia obviates one of the chief criticisms of this type of experiment, since the reactions are those to the drug administered and not those to the drug plus an anesthetic agent.

The problem of tachyphylaxis affords opportunity for much speculation. Local immunity (21), circulating antibodies (22) and cardiac depression (23) have been suggested as responsible for the phenomenon of tachyphylaxis. Rietschel (24) expressed the opinion that tachyphylaxis rests on a conversion or change of the vegetative nervous system. The fact that we have found in our experiments on ephedrine and pitressin that tachyphylaxis is produced with greater difficulty after denervation than before it lends support to the conception that the sympathetic nervous system is involved in this phenomenon but in what manner is not known.

In our experiments we have never observed definite, independent capillary contraction. We have not given attention to the swelling of the endothelial cells which Sanders, Ebert and Florey observed.

Stimulation of the peripheral end of the severed cervical sympathetic chain consistently produced a definite constriction of the arterioles in the chamber, while the results after stimulation of either auricular nerve were variable. It has been stated from the work of Meltzer and Meltzer that the vasomotor supply of the central artery in most instances comes largely from the cervical sympathetic chain. Since most of the blood vessels in the chamber usually grow in from the central artery (25) it seems logical to assume that these new vessels should be supplied by the same nerve fibers which supply the central artery. This might explain the consistent results obtained by stimulation of the cervical sympathetic chain. In those instances in which stimulation of the auricular nerves resulted in definite constriction of the arterioles, it can likewise be concluded that the arterioles received at least a part of their vasomotor supply through the auricular nerves and it may be inferred that the central artery did likewise.

SUMMARY AND CONCLUSIONS

A study of the reactions of the minute blood vessels seen in the transparent chambers inserted in the ears of albino rabbits has been presented. The response of these blood vessels to epinephrine, ephedrine, pitressin and ergotoxine has been recorded for innervated and denervated ears. Observations were also made on the effect of stimulation of the auricular and cervical sympathetic nerves on these blood vessels. Each animal was grossly examined at necropsy for evidence of regeneration of nerves.

The following conclusions were drawn:

1. The tissue grown into a transparent chamber inserted in the rabbit's ear is entirely suitable for physiologic studies.

2. The response to epinephrine was a consistent constriction of the arterioles accompanied by slight narrowing of the venules.

3. Ephedrine, in general, produced the same response as epinephrine. There was evidence of refractoriness to repeated doses of ephedrine.

4. Pitressin in the dose used (0.1 pressor unit per kilogram of body weight) had the most pronounced vasoconstrictor action. There was, as with ephedrine, refractoriness to repeated doses of pitressin.

5. Ergotoxine blocked the vasoconstrictor action of epinephrine.

6. Stimulation of the cervical sympathetic chain produced a constriction of the arterioles in the window more consistently than did stimulation of the dorsal or great auricular nerves.

7. The denervated vessels in the chamber became hypersensitive to epinephrine and regained their "tone" after denervation. Suggestions have been made as to the mechanism involved.

8. The blocking action of ergotoxine and the phenomenon of tachyphylaxis are apparently somewhat altered by denervation.

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THE ELECTRIC POTENTIALS OF THE HUMAN SMALL INTESTINE[1]

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Rosenblueth and his collaborators investigated the electric potentials of smooth muscle. They utilized the nictitating membranes, pilomotor muscles, uterus and urinary bladders of cats. Early studies by Rosenblueth and Rioch (1) in 1933 revealed the electrical activity of smooth muscle divisible into two phases—an initial potential which precedes contraction and a delayed potential simultaneous with the contraction. In a later publication Lambert and Rosenbleuth (2) in 1935 were able to subdivide still further the potentials of isolated preparations. Three phases were delimited—potential I, the fast, initial monophasic potential which precedes contraction; potential II, larger, slower mono- or diphasic potentials, either preceding or occurring simultaneously with the onset of contraction; potential III, consisting of two parts, IIIb, a slow potential lasting for the duration of the contraction, and IIIa, superimposed slow potentials, of shorter duration. In a subsequent publication Rosenblueth, Davis and Rempel (3) in 1936 demonstrated that potential I is not a propagated disturbance, is probably produced by the chemical mediator, and that the electric potential of neighboring cells may add in series.

Berkson (4), in 1933, using a string galvanometer, recorded relatively slow potentials arising from the small intestine of experimental animals. These potentials could not be correlated with contractions, and he considered them artefacts. Bozler (5) in 1938 studied the action potentials of strips of small intestine of experimental animals. During relaxation he could detect no electrical activity. Stimulation produced repetitive discharges and on rhythmic movements bursts of impulses appeared. Later Bozler (6) in 1939 isolated loops of small bowel in animals and found each contraction accompanied by repetitive discharges, each pendular movement accompanied by a discharge of 3 to 15 spikes, and in the guinea pig a characteristic frequency of 1 or sometimes several spikes occurring at an average of every 1 to 1.2 seconds during resting phases.

The experiments described in this paper were conducted in order to determine whether or not action potentials could be elicited from the intact human intestine and, if so, whether these potentials could be correlated with mechanical evidence of contraction, and with the electric potentials of smooth muscle as determined in animal experiments.

METHODS. A total of five patients was studied. Four were normals and one had a well-established ileostomy.

For recording from the patient with the ileostomy, Ag·AgCl electrodes were

[1] Aided in part by a grant from E. H. Brewer Company, Inc., Worcester, Mass.

placed approximately ½ cm. apart, directly on the mucosal surface, and parallel to the long axis of the ileum. Contractions were observed instead of being measured mechanically.

The normal patients were studied by intubation. The tube was single-lumened enclosing two no. 32 enamelled, copper wires. Electrode bars were soldered to the proximal ends of the wires.

The distal end of the tube was perforated to permit inflation of the balloon. The wires were brought through the end of the tube and electrodes of solder affixed, filed smooth to a diameter of approximately 4 mm. The lengths of protruding wire were adjusted to 3 and 5 cm. A solid, rubber-covered tip was inserted and fixed in place. A balloon of condom rubber, approximately 6 cm. long and adjusted to hold 50 cc. of air without stretching, was tied in place.

The electrodes were then sewed 2 cm. apart onto ½ inch cotton tape. The tape was adjusted and tied so that inflation of the balloon kept it taut. This assured as nearly a perfect contact with the intestinal wall as possible during the studies. All connections were tested to prevent loss of air.

The tube was introduced into the small intestine by the method of Miller and Abbott (7) and under fluoroscopic guidance it was permitted to progress until it had passed the ligament of Treitz. Since the tube was stiffened by the wires, in most instances, it progressed very little from this point. A ground electrode was attached to the skin of the abdomen over the region of the tip of the tube. All recordings were made by a 3 channel, condenser coupled amplifier with ink writers (Grass).

The proximal end of the tube was connected to a tambour with a vertical-writing pen. The membrane of the tambour was selected to be relatively resistant so that its excursions did not interfere with the writing pens. The balloon was inflated with 50 cc. of air through a T-tube. The air was measured at intervals throughout the experiment to assure inflation and detect air leaks. The pen was aligned with those of the Grass oscillograph and continuous electrical and manometric records were made for periods of 2 to 3 hours.

Results. In the patient with the ileostomy, whenever a contraction was visually apparent an electrical disturbance was evident. This disturbance consisted of fast potentials at the inception, followed quickly by slow activity. The base line shift indicated a very slow potential similar to IIIb described by Rosenblueth and his collaborators. Superimposed upon this potential was other slow activity but intermediate in frequency between the initial spikes and base line sway. This base line shift was either mono- or diphasic (fig. 1).

With the solder disc electrodes attached to the balloon in the jejunum a similar type of activity was obtained. In these records, however, the electrocardiogram appeared constantly (fig. 2). The rapid initial potentials are not as clearly evident in records taken in this manner and the faster potentials are frequently dispersed for a considerable distance along the record. This may well be due to the larger diameter of the solder electrodes as compared with the Ag·AgCl electrodes and also to the greater distance between the former. It is conceivable that initial potentials from more neighboring fibres are available for recording.

A polyphasic nature of the base line shift is more easily discernible in records obtained with the solder disc electrodes.

Correlation of the electric potentials with the mechanogram—a tambour in continuity with the tube and inflated balloon—reveals that the electrical activity is evidenced for an appreciable time before mechanical contraction is recorded (fig. 2). This time interval was constant for a single experiment and often was as much as 1.5 to 2 seconds.

The electrical activity not only preceded but usually did not persist for the duration of the mechanical evidence. Indeed, the onset of another contraction could at times be discerned electrically before the mechanogram had reached the base line. When the record is obtained at a faster speed, the difference

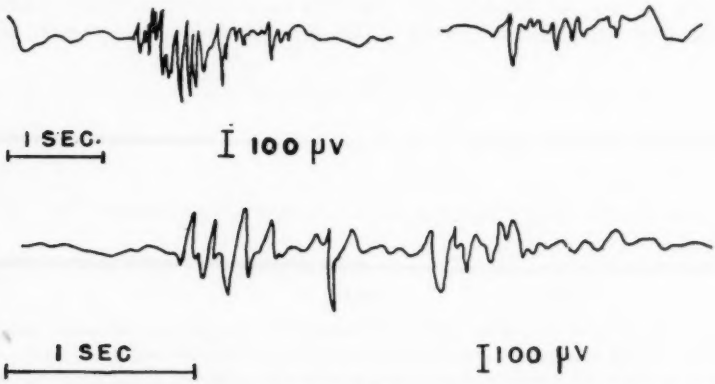

Fig. 1. Records obtained by means of Ag·AgCl electrodes from mucosal surface of small intestine exposed by old ileostomy. Upper two tracings at slower speed than lower tracing. Each record taken during contraction. Onset of activity is with fast frequency spikes followed by slow base line shift with superimposed intermediate activity.

between the three types of activity—the initial rapid spikes, the base line shift and the intermediate slow waves—is well exemplified (fig. 2).

Discussion. Electrical potentials were obtained from the intact human intestine by means of electrodes introduced via intubation. The activity thus obtained shows considerable similarity to that described for isolated preparations of smooth muscle. That discrepancies should be present is understandable in view of the complexity of this preparation. In these experiments, not only were there many fibres in the area studied, but these fibres being both longitudinal and circular were at right angles to each other. Nevertheless the same essential features are discernible, namely, rapid, initial potentials succeeded by slow waves consisting of a base line shift with superimposed intermediate slow activity. The potential II described by Lambert and Rosenblueth cannot be demarcated in these studies. Indeed potential I, as has been indicated, is complex. This complexity of faster frequencies with a spread into the sphere of the slower

activity is understandable in view of the subsequent activation of adjacent muscle fibres.

In the resting phases, that is, when mechanical evidence of contraction is lacking, there is no indication of electrical activity. This is in contradistinction to Bozler's observations in the guinea pig.

It has been noted that the electric potentials preceded the mechanical evidence of contraction. While this is not surprising, an interval of time of this magnitude (1.5–2 sec.) had not been anticipated. The explanation for this time lag probably includes the size of the balloon, the placement of the electrodes, and the inertia of the mechanical system.

The precedence of electrical activity would indicate that what has been recorded is not artefact. Moreover, movement of the electrodes over the small intestinal surface, in the ileostomy experiment, produced activity clearly discernible from the activity shown. The activity produced by such artefact

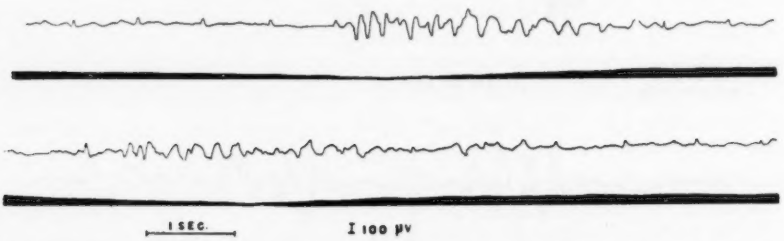

Fig. 2. Continuous record by intubation technique through two relatively short contractions. Upper tracings are electric potentials of small intestine and the E. K. G. Lower tracings are from the tambour and record mechanical evidence of contraction. Record shows onset of electrical activity before the mechanical evidence. The three phases of the electrical activity are evident.

consisted of slow waves to the extent of amplification with interspersed spikes resembling electrode "pops". In intubation experiments, artefacts were encountered only during a violent contraction often associated with cramping and borborygmus, and in these instances the tube was later found by fluoroscopy to have shifted position. However, even in these instances the artefacts were preceded by distinct activity such as shown in the illustrations.

CONCLUSIONS

1. Electric potentials can be obtained from the intact human small intestine or the small intestine previously exteriorised.

2. These electric potentials correlate with the mechanical evidence of contraction.

3. In essential respects these electric potentials are similar to the activity of isolated preparations of smooth muscle.

4. The similarity consists of fast initial spikes followed by long base line shifts with superimposed intermediate activity.

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PLASMA RETENTION, URINARY EXCRETION AND EFFECT UPON CIRCULATING TOTAL RED CELL VOLUME OF INTRAVENOUS GELATIN IN NORMAL DOGS[1]

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In a previous paper (1) it was shown that intestinal capillaries which were injured sufficiently to permit the partial or complete passage of serum proteins through their walls allow the passage of only 35 to 60 per cent of plasma gelatin. In view of this fact the rate of disappearance of gelatin from plasma is of interest especially with reference to the use of gelatin solutions as blood substitutes. Amberson (2) cites data reported by various authors which show that infused gelatin had completely disappeared from the blood in from 2 to 40 hours after its infusion in normal animals. There was considerable difference of opinion regarding the rate of its disappearance, probably because indirect methods for determining its concentration in the blood were used.

The purpose of the investigations reported here was the study of: *a*, the disappearance of intravenously injected gelatin-saline solutions from the blood after single and repeated injections in normal dogs, and the excretion of gelatin in the urine; *b*, the effect of intravenously injected gelatin upon the total red cell volume.

PROCEDURE AND METHODS. *Disappearance from the blood and the excretion of gelatin.* Unanesthetized dogs were used. A plasma volume determination was done using the method of Gibson and Evelyn (3) and blood was drawn for analysis. The bladder was catheterized and emptied. The animals were then given an intravenous injection of gelatin-saline solution (4 to 7 per cent) equal to 1 per cent of the body weight. In the case of 4 dogs blood was drawn for analysis and plasma volume determinations were made at intervals of ½, 6, 24, 48 and 72 hours. All urine was collected for analysis at intervals of 6, 24, 48 and 72 hours, the period of collection being terminated always by catheterization. Four other dogs received gelatin-saline injections at intervals of 24 hours for 4 days. Blood was drawn for analysis and plasma volume determinations were done just prior to these injections. All urine was collected for analysis at intervals of 24 hours and each period of collection was again terminated by catheterization.

The serum analyses were done according to a previously published procedure (1). It was necessary to devise a procedure for the determination of gelatin in urine. It was found that 5 per cent trichloracetic acid will not precipitate gelatin from urine, but it will precipitate other proteins which may be present. Gelatin is precipitated by tungstic acid. From 0.2 to 6.0 ml. of urine are pipet-

[1] Aided in part by a grant from the Knox Gelatine Company. The gelatin was furnished by Knox Gelatine Company, lot number B 78-1's.

ted into a 60 ml. centrifuge tube used in the Sorvall angle centrifuge, the volume of urine to be used being estimated by a preliminary precipitation. The total volume is brought to 9.0 ml. by the addition of distilled water; 0.5 ml. of 10 per cent sodium tungstate and 0.7 ml. of $\frac{2}{3}$ N sulphuric acid are added. In some cases it is necessary to add up to a total of 1.0 ml. of sulphuric acid before precipitation is complete. The tubes are allowed to stand for 15 or 20 minutes if flocculation does not occur immediately and are then centrifuged for 15 minutes or until the supernatant fluid is clear. The completeness of precipitation is checked by adding 1 drop of $\frac{2}{3}$ N sulphuric acid to the clear supernatant fluid. If more precipitate forms, several drops of the acid are added, and the tube is centrifuged again. The fluid is then decanted and the precipitate is washed with 4 ml. of a solution made by mixing 1 part of 10 per cent sodium tungstate, 1 part of $\frac{2}{3}$ N sulphuric acid and 18 parts of distilled water. The tube is centrifuged and decanted, and this washing process is again repeated. Decantation causes no appreciable loss of precipitate, which was ascertained by using an asbestos plug filter in some cases.

The nitrogen in the precipitate is determined according to the method of Van Slyke (4) and Van Slyke and Kugel (5). After digestion and neutralization the solution is transferred with washing to a 25 milliliter volumetric flask and brought up to volume with distilled water. Ten ml. aliquots of the solution are used for the manometric determination of nitrogen, but in some cases it is necessary to use greater or smaller aliquots depending upon the quantity of nitrogen present. The nitrogen values are converted to gelatin values by the usual factor 6.25.

All urine samples were tested for the presence of proteins other than gelatin by precipitation in a 5 per cent trichloracetic acid solution. In no case was protein other than gelatin found.

All determinations were done in duplicate, and those not checking within 5 per cent were repeated.

The effects of gelatin on the total red cell volume. Observations on the total red cell volume were made using the method of Gibson and Evelyn (3). Unanesthetized dogs were used. All blood samples were drawn from the jugular or femoral vein without stasis. Control determinations of total red cell volumes were made after which the animal was given intravenously a quantity of gelatin solution equal to 1 per cent of the body weight. Some animals received a single injection of 6 to 7 per cent gelatin after which the red cell volume determinations were made at intervals of $\frac{1}{2}$, 6, 24, 48 and 72 hours. Other animals received approximately 4 per cent gelatin solutions injected at 24 hour intervals for 4 days, the total red cell volume being determined prior to each injection. In some of these animals injections of 6 per cent gelatin solutions were continued for 7 or 8 days after which the total red cell volume was again determined.

RESULTS AND DISCUSSION. In order to test the accuracy of the urinary gelatin determinations, a known quantity of gelatin was added to urine which had been collected during some of the preliminary experiments and was known to contain gelatin. The results are given in table 1. It will be seen that the maximum variations from the theoretical concentrations is less than 7 per cent.

Disappearance from the blood and excretion of gelatin. Complete data obtained

following a single injection of a gelatin solution in a typical experiment are given in table 2, and the data on the disappearance of gelatin from the plasma and its excretion in the 4 animals in this group are summarized in table 4.

Approximately 50 per cent of the injected gelatin had disappeared from the plasma within 30 minutes after injection, and by the end of 72 hours only traces of gelatin remained in the plasma. This means that gelatin began leaving the blood soon after its injection and left rather rapidly. However, only 23 to 35

TABLE 1

Recovery of gelatin added to urine

TOTAL GELATIN CONCENTRATION	DETERMINED ORIGINAL GELATIN CONCENTRATION	CONCENTRATION OF ADDED GELATIN	
		Found	Theoretical
gram per cent	*gram per cent*	*gram per cent*	*gram per cent*
0.67	0.15	0.52	0.55
0.67	0.15	0.52	0.55
0.83	0.27	0.56	0.55
0.84	0.27	0.57	0.55
1.61	0.80	0.81	0.87
1.63	0.80	0.83	0.87

TABLE 2

Disappearance of gelatin from blood following a single intravenous injection and its excretion in urine. Dog 3. Received 10.04 grams gelatin as a 7 per cent solution

HOURS AFTER GELATIN INJECTION	PLASMA VOLUME	SERUM PROTEIN CONC.	SERUM GELATIN CONC.	SERUM N.P.N.	TOTAL SERUM PROTEIN	TOTAL SERUM GELATIN	GELATIN LOST FROM PLASMA	HOURS AFTER GELATIN INJECTION	URINE VOLUME	URINE GELATIN CONC.	TOTAL URINE GELATIN
	ml.	*gram per cent*	*gram per cent*	*mgm. per cent*	*gram*	*gram*	*gram**		*ml.*	*gram per cent*	*gram**
Control	600	6.21		33.5	37.26						
½	773	4.97	0.71	35.9	38.42	5.49	4.55	0–6	193	0.29	0.56
6	678	5.22	0.43	35.7	35.39	2.92	7.12				
24	678	5.38	0.23	37.7	36.48	1.56	8.48	6–24	217	0.70	2.08
48	657	5.72	0.16	23.3	37.58	1.05	8.99	24–48	175	0.22	2.47
72	760	5.91	0	26.3	44.92		10.04	48–72	297	0.11	2.80

* Cumulative values.

per cent of that which left the plasma was excreted in the urine. Whether the gelatin not accounted for in the urine is stored or metabolized is not known. Brunschwig et al. (6) have obtained evidence which suggests that at least a portion of intravenously injected gelatin is metabolized.

The excretory rate of gelatin diminished as the serum concentration decreased, as one would expect. The serum gelatin concentrations given in table 2 are quite representative of those in the other dogs of this group.

The gelatin induced a diuresis during the first 6 hours following its injection. During this period the hourly urinary volume is about 100 per cent greater than subsequent hourly excretion rates. This confirms unpublished evidence ob-

tained on anesthetized dogs in which it was found that the urinary volume was from 2 to 18 times greater per unit of time after gelatin than during the control period.

TABLE 3

Disappearance of gelatin from blood during repeated injections and its excretion in urine. Dog 7. Received 4 per cent gelatin solution

DAYS	GELATIN INJECTED	PLASMA VOLUME	SERUM PROTEIN CONC.	SERUM GELATIN CONC.	SERUM N.P.N.	TOTAL SERUM PROTEIN	TOTAL SERUM GELATIN	GELATIN LOST FROM PLASMA	URINE VOLUME	URINE GELATIN CONC.	TOTAL URINE GELATIN
	*gram**	*ml.*	*gram per cent*	*gram per cent*	*mgm. per cent*	*gram*	*gram**	*gram**	*ml.*	*gram per cent*	*gram**
Control		800	7.05		24.7	56.4					
1†	8.86	891	6.52	0.18	35.9	58.1	1.60	7.26	880	0.12	1.06
2	17.72	874	6.30	0.37	24.9	55.1	3.23	14.49	525	0.53	3.84
3	26.39	874	6.05	0.47	22.0	52.9	4.11	22.28	700	0.40	6.64
4	35.06	908	5.99	0.32	45.5	54.4	2.91	32.15	405	0.66	9.31

* Cumulative values.

† All samples were drawn at the end of a 24 hour period.

TABLE 4

Summary of data on gelatin disappearance from the blood and its excretion in the urine

DOG	SINGLE INJECTION OF 6 TO 7 PER CENT GELATIN SOLUTION					DOG	REPEATED INJECTIONS OF 4 PER CENT GELATIN SOLUTION			
	Hours after injection						Days			
	½	6	24	48	72		1	2	3	4
1 A*		54.7	81.3		95.5	5 A	85.3	78.4	91.0	93.7
B		19.7	21.7		26.9	B	31.6	31.9	30.5	33.0
2 A	55.7	71.1	86.7	96.7	98.8	6 A	91.9	81.1	90.6	85.3
B		17.0	28.8	33.3	35.0	B	25.7	33.0	31.5	33.3
3 A	45.3	70.9	84.5	89.5	100	7 A	81.9	81.8	84.4	91.7
B		7.9	24.5	27.5	27.9	B	14.6	26.5	29.8	29.0
4 A	47.6	64.7	84.3	93.0	98.2	8 A	80.9	82.1	86.3	85.8
B		17.1	20.3	22.2	22.7	B	24.4	32.1	32.6	35.1

* A: Per cent of total injected gelatin lost from plasma. B: Per cent of total gelatin lost from plasma found in urine.

In one dog (table 2), the plasma volume increased and remained elevated for 3 days. In all of the animals the plasma volume was somewhat elevated 30 minutes after the injection of gelatin, but in 3 dogs it had returned to approximately the control level in 6 hours.

Data obtained with repeated gelatin injections in a typical experiment are given in table 3 and a summary of data is given in table 4.

There is some increase in the serum gelatin concentration and in the total serum gelatin with repeated injections, although the per cent of the injected gelatin which left the plasma is of the same order as that found with a single injection. The fraction of the gelatin leaving the plasma and found in the urine was only slightly greater than with a single injection. In the normal dog there is little tendency for the gelatin to accumulate in the blood. When given at 24 hour intervals the dog is able to store or metabolize gelatin with the same degree of efficiency as after a single injection.

In contrast with the single injection experiments there was a consistent increase in the plasma volume with repeated injections. On the average this was

TABLE 5

The effect of a single injection of gelatin solutions on the total red cell volume

DOG	CONCENTRATION OF GELATIN SOLUTION INJECTED		HOURS AFTER GELATIN INJECTION					
			Control	½	6	24	48	72
	gram per cent							
1	6.30	Hematocrit reading	44.6		38.3		38.3	35.2
		Red cell volume (ml.)	611		458		451	395
		Per cent change from control value in R.C. volume			−25		−26	−35
2	6.84	Hematocrit reading	46.0	34.8	37.0	36.6	35.6	31.2
		Red cell volume (ml.)	273	186	197	179	189	144
		Per cent change from control value in R.C. volume		−32	−28	−34	−31	−47
3	7.17	Hematocrit reading	50.8	35.8	36.4	37.3	37.8	36.3
		Red cell volume (ml.)	620	431	388	403	399	433
		Per cent change from control value in R.C. volume		−31	−37	−35	−36	−30

a 10 per cent increase and it was maintained throughout the experimental period.

There was a considerable decrease in the serum protein concentration, but the changes in the total serum protein were not significant. This can be explained by the increase in the plasma volume.

The observations reported here show that in the normal dog intravenously administered gelatin leaves the plasma at a rather rapid rate. This raises the question as to whether gelatin solutions would be very effective as a blood substitute. Although the evidence is not very extensive, it has been shown in hypoproteinemic dogs and humans (7, 8) that the transfused plasma and serum proteins leave the blood stream very rapidly. It is possible that in shock the infused gelatin will not leave the blood stream as rapidly as in the case of normal dogs. In the case of 3 dogs in shock which received gelatin we found (unpub-

lished data) that after 21 hours the gelatin concentration was 48 per cent the concentration immediately after injection, after 19 hours 34 per cent and after 24 hours 84 per cent. In the series reported here the average gelatin concentration 24 hours after injection is approximately 30 per cent that found ½ hour after injection.

The effect of gelatin upon the total red cell volume. The effect of single injections of gelatin solutions (6.3 to 7.17 per cent) on the total red cell volume will be found

TABLE 6

The effect of repeated injections of gelatin solutions on the total red cell volume

DOG	DAYS	CONCENTRATION OF GELATIN SOLUTION INJECTED	HEMATOCRIT READING	TOTAL RED CELL VOLUME	CHANGE FROM CONTROL VALUE IN R.C. VOLUME	DOG	DAYS	CONCENTRATION OF GELATIN SOLUTION INJECTED	HEMATOCRIT READING	TOTAL RED CELL VOLUME	CHANGE FROM CONTROL VALUE IN R.C. VOLUME
		gram per cent		*ml.*	*per cent*			*gram per cent*		*ml.*	*per cent*
1	Control	3.81	53.0	986		4	Control	3.92	49.8	717	
	1	3.87	52.5	900	−9		1	3.92	40.8	560	−22
	2	3.87	47.5	837	−15		2	3.92	37.7	476	−34
	3	3.87	45.1	774	−22		3	3.92	36.8	458	−36
	4		45.7	736	−25		4	6†	34.4	427	−41
							4–11	6†	30.3	365	−49*
2	Control	4.35	48.3	586		5	Control	4.12	48.5	744	
	1	4.35	44.7	658	+12		1	4.12	41.5	632	−15
	2	4.23	39.3	527	−10		2	4.03	42.5	646	−13
	3	4.23	38.5	492	−16		3	4.03	40.7	600	−19
	4		37.8	486	−17		4	6†	36.9	531	−29
							4–12	6†	35.0	396	−47*
3	Control	4.35	44.2	680		6	Control	4.12	52.4	929	
	1	4.35	41.1	711	+5		1	4.12	50.5	945	+2
	2	4.23	40.0	583	−14		2	4.03	51.1	966	+4
	3	4.23	37.3	560	−18		3	4.03	44.8	811	−13
	4		36.4	539	−21		4	6†	41.5	668	−28
							4–12	6†	38.7	573	−38*

* Determinations of the total red cell volume done 24 hours after the last injection.
† Approximately 6 per cent by weight allowing for the estimated water content.

recorded in table 5. There was an almost immediate decrease in the total red cell volume which remained practically unchanged for 48 hours. In two cases there was a further decrease after 72 hours.

The effect of repeated gelatin injections will be found in table 6. In these experiments the concentration of the injected gelatin was approximately 4 per cent in contrast with the above single injections. These injections were given daily for 4 days. In three animals there was a slight decrease at the end of 24 hours while in the others there was a slight increase in the total red cell volume.

In all animals, except no. 6, the decrease had become evident by the end of the second day. In most instances as the injections proceeded the diminution in total red cell volume was not directly proportional to the amount of gelatin injected. In three animals (dogs 4, 5 and 6) the injections were continued for 7 or 8 days beyond the usual 4 day period but with approximately 6 per cent gelatin instead of 4 per cent gelatin. At the end of this period there was a further decrease in the total red cell volume. However, this decrease was less than one might expect from the results with single injections of 6 per cent gelatin as will be seen by comparing the data in table 5 with those on dogs 4, 5 and 6 in table 6.

It is quite evident that the gelatin which was used in these experiments causes a decrease in the circulating total red cell volume. When the animals received repeated injections of 4 per cent gelatin the decrease was not as marked at first as with 6 to 7 per cent solutions. After 12 daily injections of gelatin the animals were not critically anemic. However, this effect must be kept in mind in interpreting results obtained by transfusion with gelatin solutions after hemorrhage, since the mortality found may be due to the anemia rather than to an inadequate maintenance of blood volume.

The cause for the decrease in total red cell volume following injection of gelatin solutions is not known. It is possible that the decrease may be due to hemolysis, or to increased phagocytosis of the red cells by the reticulo-endothelial system. Another possibility is that the size of individual erythrocytes is decreased. In three animals there was no significant difference between the decrease in the erythrocyte count and the hematocrit reading. If the size of the individual erythrocyte were decreased one would expect to find a greater decrease in the hematocrit reading than in the erythrocyte count.

SUMMARY

1. A procedure for the determination of gelatin in urine has been described.

2. Following a single injection of a gelatin-saline solution approximately 50 per cent of the gelatin left the plasma within 30 minutes. Only traces of gelatin were present after 72 hours.

3. Following a single injection of a gelatin-saline solution from 23 to 35 per cent of the gelatin which left the plasma was found in the urine.

4. With repeated injections of gelatin-saline solutions there was some increase in the serum gelatin concentration and total amount of serum gelatin. The total amount of serum proteins was unaffected, but the serum protein concentration decreased.

5. Following a single injection of 6 to 7 per cent gelatin solutions there is a marked and almost immediate decrease in the circulating total red cell volume which persists for at least 3 days.

6. During repeated injections of 4 per cent gelatin the immediate decrease in red cell volume did not always occur, and when it did occur it was not as marked as with 6 per cent gelatin solutions. With repeated injections there is progressively less effect on the total red cell volume.

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OLFACTORY DISCRIMINATION AFTER DESTRUCTION OF THE ANTERIOR THALAMIC NUCLEI

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The survey of forebrain structures by Swann (1935) and by Ghiselli and Brown (1938) has failed to reveal any structure essential for the olfactory discrimination between oil of anise and sheep dip (creosote). Allen (1941) has criticized this work on the ground that discrimination of these odors might be mediated alternatively by olfactory or trigeminal stimulation, but it should be emphasized that Swann was unable to obtain a discriminative reaction from any animal in which the olfactory bulbs had been destroyed. It seems certain that, in the concentrations used in his experiments, there was no differential trigeminal stimulation.

In a careful series of studies Allen (1941 and earlier) has reported effects upon olfactory reactions only after destruction of the frontal lobes or of the pyriform-amygdaloid complex in dogs. He concluded in summing up his work (1941) that disturbance of the relations between the pyriform-amygdaloid complex and the isocortex results in a loss of discriminative reactions and that the conditioned reaction to a single odor (presence vs. absence of odor) is probably mediated by impulses which pass to the cortex by way of the anterior thalamic nuclei (the principal path not explored in his experiments). It is not clear from his data that he obtained a true olfactory defect. On the contrary, the similarity of symptoms after frontal lobe and pyriform lesions and the indications of occasional discrimination by animals with such lesions suggest that he was dealing with a more general deterioration involving disturbances of the process of comparison, such as have been described after frontal and temporal lesions in monkeys.

Of cerebral systems intimately associated with primary olfactory centers, only the anterior nuclei have not been systematically investigated. Their cortical field is very extensive, including probably all of the infraradial and retrosplenial areas (Lashley, 1941) and the course of the projection fibers is such that the entire field has probably never been isolated from the thalamus in any experimental animal or clinical case. The present experiments were designed to test the effects of complete interruption of the radiations of the anterior nuclei upon a previously formed olfactory discriminative habit.

CORTICAL FIELD OF THE ANTERIOR NUCLEI. Three divisions of the anterior nucleus are distinguishable in the rat; the anterodorsal, anteroventral and anteromedial. The course and termination of their radiations has been described by Lashley (1941) on the basis of retrograde degeneration. The radiation of the anterodorsal nucleus passes forward around the fornix and through the callosal

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fibers just in front of the thalamus to terminate in the Area retrosplenialis granularis. That of the anteroventral follows the same course, spreading more laterad, and terminates in the Area retrosplenialis agranularis. The radiation of the anteromedial nucleus passes forward along the mesial surface of the striatum to the Area infraradiata. The apparent course and termination of the fibers, as determined from lesions producing retrograde degeneration, are shown in figure 1. Section of the radiation results in complete disintegration of all neurons in the nucleus.

METHODS. *Animals.* Black female rats about five months old from a strain derived from a cross of albino with wild gray were used for the experiments.

Training. The apparatus used for training consisted of three small blocks of wood, each containing a well, 30 mm. in diameter and 40 mm. deep, provided

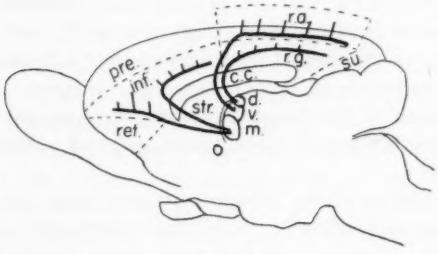

Fig. 1

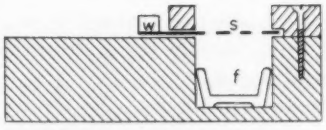

Fig. 2

Fig. 1. Diagram of the anatomic projection of the anterior nuclei upon the cerebral cortex. *d.*, anterodorsal nucleus; *v.*, anteroventral nucleus; *m.*, anteromedial nucleus; *c.c.*, corpus callosum; *inf.*, area infraradiata; *pre.*, regio praecentralis; *r.a.*, area retrosplenialis agranularis; *ret.*, regio retrobulbaris; *r.g.*, area retrosplenialis granularis; *str.*, corpus striatum; *su.*, area presubicularis. (Terminology of Rose, 1929.)

Fig. 2. Sectional sketch of one of the three training boxes. The circular well is closed by the perforated sliding cover, *s*, weighted by a small lead block, *w*. Food or cotton saturated with an odorous substance is placed in the glass dish, *f*, in the well. The rat opens the cover by clawing at the perforations above the well.

with a glass container at the bottom and with a perforated sliding metal cover which the rats could pull open (fig. 2). The blocks were impregnated and coated with paraffin so that they could be washed and recoated to remove odors. One block was placed in each of the three corners of a square restraining cage, 65 by 65 cm., and the rat admitted at the fourth. Milk-soaked bread was placed in one well and cotton with a few drops of oil of wintergreen in the others. The relative position of the blocks was varied in chance order in successive trials. The rats were given 5 to 10 trials per day until they chose the block containing bread and milk correctly in 28 or more of 30 consecutive trials (criterion of discrimination). Any attempt to open the cover of a well which did not contain food was counted as an error.

Controls. Reaction to position was controlled by chance arrangement of the relative positions in which the three blocks were placed. Non-olfactory cues

from the blocks were eliminated by regular washing and placing the food in each of the three in succession.

Oil of wintergreen is listed by Allen as a trigeminal irritant. Two controls were introduced to determine whether the rats were reacting to such stimulation and merely avoiding the wintergreen. *a.* Milk-soaked bread was added to the wells containing wintergreen after errorless performance had been established in operated animals. This led in every case to a confusion, with chance scores, showing that the animals had been reacting to the food odor and that they could detect the food odor when mixed with wintergreen. *b.* When discrimination followed the cerebral operations, the animals were subjected to a second operation involving destruction of the olfactory bulbs. Total removal of the bulbs permanently abolished the discrimination, showing that the rats were not reacting to the trigeminal component of the wintergreen stimulation.

Operation. The animals were anesthetized with ether, the skull trephined near the midline on each side. A thermocautery was thrust diagonally downward and forward through the region of the genu to a sufficient depth to pass through the striatum, to sever the radiation of the anteromedial nucleus. A shallow transverse incision to the depth of the corpus callosum was also made above the septum to interrupt the radiations of the anterodorsal and anteroventral nuclei.

Analysis of lesions. At the termination of the experiment the brains were fixed in alcohol, sectioned and stained with thionin. With two exceptions, the animals had survived long enough to permit of total degeneration of injured cells, so that the boundaries of degenerated areas were clearly defined. Sections through the thalamus were examined for retrograde degeneration and camera lucida sketches made to show the approximate amount of degeneration in each nucleus.

EXPERIMENTAL RESULTS. Eight animals were carried through training, operation and postoperative tests. All gave evidence of retention of the discriminative reaction after operation. In five of the eight destruction of the radiation was incomplete so only the records of the three cases which are crucial for the problem will be reported.

No. 1. This animal made 37 correct choices in 38 trials after 15 trials of training. She was then rested ten days and given preoperative retention tests, making no error. Operated and retested after 10 days, she gave 50 consecutive errorless trials in postoperative retention tests. The olfactory bulbs were then destroyed. Subsequent tests gave chance scores with no evidence of discrimination.

Sections showed total degeneration of the right anterodorsal, anteromedial and anteroventral nuclei. About 20 normal cells per section (less than 6 per cent of the normal number) were found in the left anterodorsal nucleus. The left anteroventral and anteromedial nuclei were totally degenerated. The nucleus reuniens was totally degenerated on both sides and the right and left dorsomedial nuclei showed some degeneration. The anterior half of the septum was destroyed and the third ventricle much distended.

No. 2. Thirty-seven of 38 trials were correct after 15 trials of training. Preoperative retention tests gave 10 consecutive errorless trials. She did not attempt to open the slides until the fourteenth day after operation, then gave 29 of 30 trials without error. The second operation, to destroy the olfactory bulbs, failed to remove the greater part of the left. Ensuing tests gave errorless performance for 3 days. The rat then developed an infection of the remaining bulb and the score fell to chance.

Sections showed total degeneration of the anterodorsal, anteroventral and anteromedial nuclei and of the nucleus reuniens on both sides. There was also extensive degeneration in the median division of the nucleus ventralis. The dorsal margin of the septum was destroyed.

No. 3. After 22 trials of training 40 errorless choices were made in 42 trials. The operation followed without the usual preoperative rest period. Following operation the animal had to be force-fed for several days and did not attempt to open the boxes until the twentieth day after operation. She then gave 39 correct responses in 42 trials. The olfactory bulbs were next destroyed. Subsequent training gave only 4 errorless in 40 trials, with no evidence of improvement.

Sections showed the anterodorsal, anteroventral, anteromedial and reuniens nuclei totally degenerated on both sides. The dorsal part of the medial division of the ventral nucleus and the mesial portion of the lateral nucleus also showed bilateral degeneration. The septum was entirely destroyed and there was slight invasion of the dorsal convexity of both hippocampal lobes.

These three cases give crucial evidence of the persistence of olfactory discrimination after interruption of the cortical radiations of the anterior nuclei. In numbers 2 and 3 all neurons of the anterior group had disappeared and in number 1 there were only a few possibly functional cells in the left anterodorsal nucleus. All of the animals discriminated accurately after the cerebral operation. Numbers 1 and 3 lost the habit after destruction of the olfactory bulbs and number 3 failed to reacquire it within twice the amount of training required for original learning. In number 2 the discrimination persisted after partial destruction of the olfactory bulbs but disappeared with infection of the remaining olfactory tissue. Thus it is clear that the discriminative reaction was mediated by the olfactory bulbs and that it was not abolished by total degeneration of all neurons in the three divisions of the anterior nucleus.

In the remaining five animals there was less extensive degeneration in the anterior nuclei, ranging from the persistence of a few normal cells in both anterodorsal nuclei to the preservation intact of one anteroventral nucleus. Their behavior was completely consistent with that of the three reported above.

Discussion. By analogy with other senses one would expect to find an olfactory area in the isocortex essential for the most highly specialized discriminative reactions, with more primitive sensorimotor reactions mediated by thalamic or other subcortical mechanisms. The anatomic connections of the anterior thalamic nuclei have led Herrick (1931) and Kappers, Huber and Crosby (1936) to ascribe to these structures the correlation of olfactory with general somatic reactions. The cortical field of the anterior nuclei is extensive; equal

in area to the visual, auditory, or somesthetic fields, and has as yet no demonstrated function. There is therefore good reason to anticipate olfactory loss after total elimination of this system. The present experiments, however, have not revealed any disturbance of olfactory function after section of the radiations and total degeneration of the cells of the anterior nuclei. They suggest that the system is not involved in olfactory functions. It should be pointed out, however, that the stimuli used were intense and perhaps simple. The olfactory apparatus of macrosmatic animals seems to be specialized for differentiation of organic odors to which man is relatively insensitive, and experiments with such substances as stimuli must be carried out before final conclusions concerning the olfactory functions of the anterior nuclear system are justified.

SUMMARY

Olfactory discrimination of rats was tested after interruption of the cortical radiations of the anterior group of nuclei. Discrimination between the odors of oil of wintergreen and of bread and milk was not disturbed by total bilateral degeneration of the anterodorsal, anteroventral and anteromedial nuclei, with additional involvement of the septum. Removal of the olfactory bulbs permanently abolished the discrimination, showing that the reaction was based upon olfactory and not trigeminal stimulation.

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THE EFFECTS OF OCCLUSION OF EXPERIMENTAL CHRONIC PATENT DUCTUS ARTERIOSUS ON THE CARDIAC OUTPUT, PULSE AND BLOOD PRESSURE OF DOGS[1]

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The possibility of the surgical repair of patent ductus arteriosus (botalli) in human beings, first successfully demonstrated by Gross and Hubbard (5), has stimulated renewed interest in this condition. When a patent ductus arteriosus is present, the abnormal shunting of blood from the aorta to the pulmonary artery leads to a train of pathologic changes particularly in the heart and the large arteries. It is not the purpose of this paper to discuss arteriovenous aneurysms in detail but there are two important differences between patent ductus arteriosus and peripheral arteriovenous fistula to which attention may be called: 1, the output of the right and that of the left ventricle is unequal in the presence of a shunt between the aorta and pulmonary artery but is equal in peripheral arteriovenous fistula, and 2, in patent ductus arteriosus the increased volume flow is in the pulmonary circulation alone while in arteriovenous fistula an increased volume flow occurs in both the systemic and pulmonary vessels. A condition similar to patent ductus arteriosus in man may be produced experimentally in dogs by lateral anastomosis between the aorta and the left pulmonary artery. It may also be produced by end-to-side anastomosis of the left subclavian artery to the main or left pulmonary artery (2).

The purpose of the present experiments was to determine the immediate effect of occlusion of a long standing experimental patent ductus arteriosus on the blood pressure, pulse and cardiac output of the right ventricle and of the left ventricle in dogs. Additional experiments dealt with the immediate effect on the cardiac output of the right ventricle only of an experimental ductus.

METHOD. Large dogs fed on kennel ration supplemented with meat were used in all instances. Food was withheld before any procedure was performed. The operations were performed under sodium pentobarbital anesthesia administered intravenously with air given intratracheally under positive pressure. The fourth or fifth left intercostal space was opened. The vessels were occluded by rubber-shod clamps and a side-to-side anastomosis was made between the aorta and the left pulmonary artery. In a similar manner an end-to-side anastomosis was made between the left subclavian and the left pulmonary artery after ligation distally of the left subclavian artery.

Preliminary determination of the cardiac output was done without anesthesia one or more days before the operation in most of the cases. A grain of morphine was administered and the consumption of oxygen was determined by means of the Benedict-Roth spirometer. Samples of blood were obtained under oil

[1] Aided by a grant from the Christine Breon Fund.

from the right ventricle and from the left ventricle or femoral artery. Potassium oxalate was the anticoagulant. The oxygen consumption was determined before each operation with the animals under anesthesia and again after the operation was completed and the chest closed. While the chest was open, specimens of blood were obtained from the aorta, from the pulmonary artery at various sites, and sometimes from the right ventricle. The oxygen content of the blood was determined manometrically in duplicate on samples of 2 cc. each using the Van Slyke-Niell apparatus (10). The output of the right ventricle[2] was calculated by the Fick principle (3). In each instance where it was possible the output of the left ventricle was also calculated using the oxygen content of mixed aortic and pulmonary artery blood taken from the pulmonary artery in the Fick formula.

The blood pressure was obtained by inserting into an artery a needle connected with a mercury manometer. This gives a determination near the mean blood pressure.

RESULTS. Eight experiments were performed on seven dogs. In five experiments the animals were maintained for a long period of time and, in three, only the immediate effects of an experimental patent ductus arteriosus were observed.

Following the establishment of a fistula between the aorta and pulmonary artery a turbulent stream of blood from the aorta flowed into the pulmonary artery creating a loud murmur and a thrill so pronounced that it vibrated strongly against the palpating finger. The pulse increased in rate and became collapsing in type, resembling the Corrigan pulse of aortic insufficiency. A capillary pulse was observed in the extremities and a pistol shot sound could be heard over the femoral arteries. The murmur was loud, continuous and machinery-like, with systolic accentuation, best heard posteriorly in the second intercostal space, with the animal lying in a supine position.

In five experiments a fistula between the aorta and pulmonary artery was maintained for 48, 49, 90, 903 and 1109 days respectively. All but one of the animals (no. 2) had an arterio-arterial opening established by side-to-side anastomosis between the aorta and the left pulmonary artery. In no. 2 an end-to-side anastomosis between the left subclavian and the left pulmonary artery was performed. One animal (no. 4) killed three years after operation had the largest persistent opening into the pulmonary artery, measuring 4 by 5.5 mm., as well

[2] When the cardiac output is determined by the Fick principle in normal dogs the outputs of the right and left ventricles are equal and the determination is considered to be that of either ventricle. When a ductus arteriosus is present, however, the output of the left ventricle is necessarily greater than that of the right ventricle since the left ventricle must expel not only the peripheral blood flow (which returns to the right auricle and ventricle and constitutes the right ventricle output) but also that portion of the left ventricle output which passes through the ductus into the pulmonary circulation and returns to the left auricle and ventricle to be expelled again. When the cardiac output in subjects with a patent ductus is determined in the usual manner by the Fick principle using mixed blood from the right ventricle and arterial blood the value obtained is the output of the right ventricle. To determine the cardiac output of the left ventricle in these subjects by the Fick principle, mixed blood must be obtained from the pulmonary artery.

TABLE 1

Effects of occlusion of chronic experimental ductus arteriosus

DOG NO.	DAYS DUCTUS PRESENT	BODY WT.	PROCEDURE	SOURCE OF BLOOD SAMPLES	OXYGEN CONTENT	A-V OXYGEN DIFFERENCE	CARDIAC OUTPUT			PER CENT OF C.O. FLOWING THROUGH DUCTUS	PER CENT REDUCTION IN C.O. AFTER OCCLUSION OF DUCTUS	OXYGEN CONSUMPTION
							Left and right ventricle equal	Left ventricle output	Right ventricle output			
		kgm.			*vol. %*	*vol. %*	*cc./min.*	*cc./min.*	*cc./min.*			*cc./min.*
1			Ductus established									
	35	20.1	Cardiac output, no anesthesia				4,475					
	42		Cardiac output, no anesthesia				3,362					
	48	20.5	Chest opened under intravenous anesthesia and blood samples obtained	Aorta	18.68							187
				Proximal pulmonary artery	17.11	1.57		11,911				
				Distal pulmonary artery	18.69							
				Right ventricle	15.32	3.36			5,505	46		
			After ductus occluded for 13 minutes blood samples obtained	Aorta	20.47							
				Pulmonary artery	9.53	10.94	1,618				85 lt.*	
				Right ventricle	10.26	10.21	1,832				66 rt.	
2			Cardiac output, no anesthesia				2,519					
		18.5	Ductus established									
	43		Cardiac output, no anesthesia				2,995					
	49	18.5	Chest opened under intravenous anesthesia	Aorta	20.97							262
				Proximal pulmonary artery	15.19	5.78			4,530			
				Distal pulmonary artery	18.72	2.25		11,700		39		
			Ductus occluded 12 minutes	Aorta	20.02						72 lt.	
				Pulmonary artery	12.12	7.90	3,316				27 rt.	
			Ductus occluded 27 minutes	Aorta	20.15						75 lt.	
				Pulmonary artery	11.11	9.04	2,900				36 rt.	
3		20.6	Ductus established									
	15		Cardiac output, no anesthesia				5,719					
	86	21.8	Cardiac output, no anesthesia				4,181					
	90	22.2	Chest opened under anesthesia	Aorta	12.60							315
				Proximal pulmonary artery	9.36	3.24			9,722			
				Distal pulmonary artery	10.99	1.61		19,570		50		
			Ductus occluded 10 minutes	Aorta	9.26						72 lt.	
				Pulmonary artery	3.79	5.47	5,556				54 rt.	
			Ductus reopened	Aorta	10.91							
				Pulmonary artery	8.29	2.62		12,023				

TABLE 1—*Concluded*

DOG NO.	DAYS DUCTUS PRESENT	BODY WT.	PROCEDURE	SOURCE OF BLOOD SAMPLES	OXYGEN CONTENT	A.V. OXYGEN DIFFERENCE	CARDIAC OUTPUT			PER CENT OF C.O. FLOWING THROUGH DUCTUS	PER CENT REDUCTION IN C.O. AFTER OCCLUSION OF DUCTUS	OXYGEN CONSUMPTION
							Left and right ventricle equal	Left ventricle output	Right ventricle output			
		kgm.			*vol. %*	*vol. %*	*cc./min.*	*cc./min.*	*cc./min.*			*cc./min.*
	901	19.9	Cardiac output, no anesthesia	Right ventricle	7.75							209
				Femoral artery	12.50	4.75	4,400					
	903	19.9	Cardiac output, intravenous anesthesia (breathing O_2)	Right ventricle	9.03							265
				Femoral artery	16.20	7.17	3,696					
			Chest opened under anesthesia	Aorta	14.70							
				Proximal pulmonary artery	6.13	8.57			3,092			
				Distal pulmonary artery	10.23	4.47		5,928		53		
			Ductus occluded for 3 minutes	Pulmonary artery	4.97						55 lt.	
				Aorta	14.91	9.94	2,666				14 rt.	
4		16.9	Ductus established									
	60	14.9	Cardiac output, no anesthesia				1,524					
	119	15.3	Cardiac output, no anesthesia				2,273					
	220	17.55	Cardiac output, no anesthesia				1,475					
	1,190	17.5	Chest opened	Aorta	16.6							139
				Proximal pulmonary artery	11.7	4.9			2,837			
				Distal pulmonary artery	14.5	2.1		6,619		43		
			Ductus occluded 5 minutes	Aorta	17.80						85 lt.	
				Pulmonary artery	4.17	13.63	1,020				64 rt.	
			Ductus reopened	Aorta	18.9							
				Pulmonary artery at ductus	18.1							
				Distal pulmonary artery	18.6							
Average										46	74 lt. 44 rt.	

* Refers to left and right ventricle output.

as the loudest murmur. The opening in no. 3 was 2 by 1.5 mm. With one exception the dogs remained in excellent condition. Number 3 gradually lost 2.1 kgm. in weight over a period of 14 months and suffered a mild degree of anemia; the hemoglobin was 80 per cent, the red blood cell count 5.44 million and the cell volume was 41 (Wintrobe). None of the dogs developed peripheral edema or symptoms of cardiac failure; the venous pressure was not elevated, and

no great deviation from normal was observed in the mean blood pressure. In these experiments the ductus was occluded from 3 to 27 minutes and measurements of the circulation were made before and after the occlusion (table 1).

Included in table 1 are the calculated values of the cardiac output of the left ventricle. These were calculated by the Fick formula from samples of

TABLE 2

Immediate effects of establishing an experimental ductus

DOG NO.	DATE	BODY WT.	PROCEDURE	SOURCE OF BLOOD SAMPLES	OXYGEN CONTENT	A.V. OXYGEN DIFFERENCE	CARDIAC OUTPUT	PER CENT INCREASE IN OXYGEN CONTENT OF BLOOD SAMPLES FROM AORTA	PER CENT INCREASE OR DECREASE IN C.O. AFTER DUCTUS MADE	OXYGEN CONSUMPTION
		kgm.			*vol. %*	*vol. %*	*cc./min.*			*cc./min.*
5	12-1 -39		Cardiac output, no anesthesia				3,836			
	12-4 -39	17.2	Chest opened under anesthesia and blood samples taken	Pulmonary artery	17.88					141
				Aorta	22.07	4.19	3,365			
			After ductus established Ductus 8 mm.	Proximal pulmonary artery	22.70					
				Distal pulmonary artery	23.25					
				Aorta	23.03			4		
				Right ventricle	17.92	5.11	3,875		13 increase	
6	12-11-39	13.0	Chest opened	Pulmonary artery	15.44					131
				Aorta	23.21	7.77	1,686			
			Ductus established Ductus 10 mm.	Proximal pulmonary artery	26.40					
				Distal pulmonary artery	25.93					
				Aorta	24.55			5		
				Right ventricle	15.72	8.83	1,484		12 decrease	
7	12-14-39	15.4	Chest opened	Pulmonary artery	15.10					112
				Aorta	20.38	5.28	2,123			
			Ductus established	Proximal pulmonary artery	15.28	5.49	2,040		4 decrease	
			Ductus 4 mm.	Distal pulmonary artery	14.85	5.92	1,892		11 decrease	
				Aorta	20.77			2		
Average								4	3.6 decrease	

mixed blood of the aorta and the pulmonary artery, obtained from the left pulmonary artery. The cardiac output of the left ventricle was roughly double that of the right ventricle and, in five experiments, the average blood flow through the ductus, expressed as per cent of the output of the left ventricle, was 46. Comparison of the oxygen content in samples of blood from the pul-

monary artery, as shown in tables 1 and 2, demonstrates that blood in the pulmonary artery is more evenly mixed in dogs in which the experimental patent ductus arteriosus has been present for a long time. Samples of blood from the pulmonary artery can be used to calculate the output of the left ventricle with more accuracy in experiments of long duration rather than in acute experiments.

In the second group, consisting of three experiments, determinations were made of the cardiac output of the right ventricle and pulse immediately before and after establishment of an experimental ductus arteriosus. The results are given in table 2. The experimental production of such a fistula is attended immediately by the following changes in the circulation: The pulse increases from 20 to 40 beats per minute. Presumably the output of the left ventricle is

TABLE 3

Effects of occlusion of experimental patent ductus arteriosus of long standing on the pulse rate and the blood pressure in the aorta and pulmonary artery (dog no. 1)

TIME	BLOOD PRESSURE		Pulse
	Aorta	Pulmonary artery	
	mm. Hg	*mm.* Hg	
2:25			130
2:33			120
2:50			110
2:55	72 proximal*	16 proximal	
2:58	68 distal	18 distal	
3:03	88 proximal†		
3:05	80 distal		105
3:08	68 proximal		
3:15			110
3:18		Occlusion of ductus	
3:20	110 proximal	16 proximal	
3:22	112 distal	16 distal	
3:30			70

* Refers to insertion of needle of blood pressure instrument proximal or distal to opening of ductus.

† Blood pressure in femoral artery was 110 mm. Hg at this time.

increased by the flow of blood through the ductus, which returns to the left auricle and ventricle. This value was not determined for the reasons given above. The output of the right ventricle was not altered significantly. The average increase in the oxygen content of the arterial blood in the three experiments was approximately 4 per cent. This increase is at least a partial manifestation, however, of an increased metabolism resulting from the anesthesia and operation.

In four experiments the pulse, and blood pressure in the aorta and pulmonary artery were recorded before and after occluding the experimental patent ductus arteriosus and the results were identical in each. The results of a typical experiment are given in table 3. It is seen from the table that occlusion of an experimental ductus is followed by a sharp rise in the blood pressure in the aorta,

a slowing of the pulse and no essential change in the blood pressure in the pulmonary artery.

The determinations of the consumption of oxygen before and immediately after operation, with the animals under anesthesia, indicate an increase in the consumption of oxygen. For this reason, the average consumption of oxygen was used in the calculations rather than the basal consumption of oxygen. Basal conditions were not approached except when the preliminary determinations of the cardiac output were obtained. The factors of anesthesia and operation served to increase the consumption of oxygen and the cardiac output, but the comparison between the output and other determinations made before and after occluding the patent ductus arteriosus is considered to indicate accurately the effects of such occlusion.

Discussion. The immediate effects of occlusion of a chronic experimental patent ductus arteriosus (tables 1 and 3) are 1, an increase in the systemic arterial blood pressure; 2, a slowing of the pulse, and 3, a decrease in the cardiac output of both ventricles. The blood pressure in the pulmonary artery changes little and remains well below one-third that in the aorta.

The immediate increase in the blood pressure in the aorta, following the closure of the ductus arteriosus necessarily follows diversion of the entire output of the left ventricle into the aorta distal to the occluded ductus.

The cardio-inhibitory reflex mechanisms mediated by the vagus nerve and carotid sinus result in a slowing of the pulse and a reduction in the minute volume output. The output of both ventricles becomes equal when the ductus arteriosus is occluded, and is reduced from 14 to 66 per cent of the original value in the case of the right ventricle and from 55 to 85 per cent of the left ventricular output. The average reduction in the cardiac output of the left ventricle was 74 per cent and that of the right ventricle 44 per cent.

A factor in the slowing of the pulse following occlusion of an experimental ductus arteriosus might be the suddenly decreased output of the left ventricle. Grollman (4), in a discussion of arteriovenous aneurysm, stated, "The bradycardia phenomenon refers to the drop in pulse rate observed on occluding the arteriovenous communication. This drop in pulse rate might be looked upon as a concomitant of a decrease in cardiac output. Lewis and Drury (9) explain it as a result of an increase in vagal tone. In view of the studies [of Harrison, Dock and Holman, (6)] the first mentioned explanation seems more likely."

The decrease in cardiac output after occlusion of the ductus is accompanied by a marked degree of deoxygenation of blood samples taken from the pulmonary artery, compared to the oxygen content of samples of blood from the aorta. The latter changes little as long as the animals receive an adequate supply of oxygen through the intratracheal catheter.

The depression of the cardiac output following occlusion of the ductus arteriosus persists for more than a few minutes as illustrated by the experiment (no. 2) in which samples of blood from the pulmonary artery and the aorta were obtained 12 and 27 minutes after the ductus was occluded by a clamp and the cardiac output determined.

There was no pronounced change in the blood pressure in the pulmonary artery following occlusion of the experimental ductus arteriosus. This is in accordance with the previous findings of Levy and Blalock (8) who connected the left pulmonary with the left subclavian artery in an end-to-end anastomosis. The blood pressure in the pulmonary artery remained at a low level, a result consistent with a low peripheral resistance in the lung.

In dogs with experimental patent ductus arteriosus blood flows from the aorta into the pulmonary artery rather than the reverse. This is demonstrated by the elevated content of oxygen in samples of blood obtained from the pulmonary artery near the ductus arteriosus, compared to the oxygen content of samples of blood from the right ventricle or pulmonary artery near the semilunar valves, when the experimental ductus arteriosus was open (tables 1 and 2). This would be expected in view of the difference in pressures in these vessels indicated in table 3.

The flow of blood through the ductus arteriosus is a considerable fraction of the output of the left ventricle. According to the determinations in man and experimental dogs, made by Eppinger, Burwell and Gross (2) by means of the Fick principle, and by the acetylene method employed by Keys (7) in man, the volume flow of blood passing through the ductus arteriosus amounts to one-third of the output of the left ventricle in average cases and to one-half in severe cases. The determinations of the flow through the ductus arteriosus in the present experiments, ranging from 39 to 53 per cent, agree with these figures.

The changes in the circulation immediately following occlusion of an experimental arterio-arterial fistula between the aorta and the pulmonary artery are of interest in connection with the surgical operation for ligation of patent ductus botalli in man. Dolley and Jones (1) emphasized the importance of first occluding the ductus arteriosus temporarily and watching for untoward effects on the cardiac action before proceeding with the ligation. Although the changes in the circulation which followed occlusion of an experimental ductus arteriosus did not result in the death of any of the experimental animals, the slowing of the pulse, increase in systemic blood pressure, decrease in cardiac output and decrease in oxygenation of mixed venous blood from the pulmonary artery are easily observed effects. The anesthesia employed should be one which insures an adequate intake of oxygen for if the subject were anoxemic or in an otherwise impaired condition, the effects of occlusion of the ductus might be harmful. In the light of these considerations, the technique advocated by Dolley and Jones is undoubtedly advisable.

SUMMARY

1. A condition similar to patent ductus botalli was produced in dogs by lateral anastomosis of the aorta to the left pulmonary artery and by end-to-side anastomosis of the left subclavian to the left pulmonary artery.

2. Studies were made of both the immediate and late effects of production of the experimental ductus arteriosus on the cardiac output, pulse and content of oxygen in the blood.

3. A study was also made of the immediate effects of occlusion of chronic experimental ductus arteriosus on the pulse, systemic blood pressure and that of the pulmonary artery and the cardiac output.

4. In the experiments of long standing the flow of blood through the experimental ductus arteriosus was calculated.

5. The significance of the findings as related to surgical ligation of patent ductus botalli in human patients is suggested.

Acknowledgment. It is a pleasure to record my obligation to Drs. R. Cressman, B. Henley, E. Perez, H. Silvani and H. B. Stephens for assistance with the operations. Dr. A. Moulay performed some of the blood oxygen determinations. Mr. Charles Ayello assisted with the experiments.

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THE SURVIVAL OF NON-ADRENALECTOMIZED RATS IN SHOCK WITH AND WITHOUT ADRENAL CORTICAL HORMONE TREATMENT

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In this study shock was induced in non-adrenalectomized rats by ligation of the two hind limbs. A comparison was made of the incidence of survival among rats which were treated with adrenal cortical extracts and steroids, and those which were untreated. The results of the study were negative.

METHODS. Normal male rats of the Sprague-Dawley strain, weighing 190 to 200 grams, were used. A short period of anesthesia was induced by the intraperitoneal injection of a solution of 18 mgm. of cyclopentenyl-allyl-barbituric acid sodium. The hair was clipped from the posterior half of the body. A ligature of strong linen thread was placed about each hind leg and held in place by four short stitches through the skin at the following points: 1, in front of the penis; 2, through the umbilical scar; 3, dorsal side, over the vertebral notch formed by the junction of the lumbar and thoracic vertebrae, and 4, just over the first caudal vertebrae. The ligatures were tightened alternately until they were as tight as possible. A second set of ligatures were then placed in the same positions but were not stitched through the skin. The ligatures were removed after two hours and thirty minutes. By this time the animals had begun to recover from the anesthetic. Food and water were withheld. At the time the ligatures were removed, the limbs were greatly swollen and congested with blood. Although the swelling and congestion were relieved to some extent following removal of the ligatures, the animals gradually lost the appearance of vigor and became prostrated. The incidence of survival for 24 hours was the criterion used to judge the therapy.

The adrenal cortical extract was prepared from beef adrenal glands. In experiment 1, each dose of extract was administered in 1.0 cc. of physiologic saline and each dose of the crystalline compounds was administered in 0.5 cc. of sesame oil. In experiment 2, an adrenal cortical extract, from which all traces of epinephrine had been removed, was administered in 0.5 cc. of sesame oil per dose. A control solution was prepared by dissolving physiologically inert material from adrenal extracts in sesame oil so that its appearance matched that of the active extract. The control animals received 0.5 cc. of this solution per dose. All test substances were given by subcutaneous injection at the time the legs were ligated and again six hours later.

The selection and matching of the rats with the test substances was by the following procedure: For experiment 1, a pack of cards, containing all the possible matchings of test substances with numbers from 1 to 5, was thoroughly shuffled. On each day a group of 5 rats were subjected to ligation of the limbs

and were then numbered from 1 to 5. A card was drawn from the pack and the test substances were administered according to the matchings on the card. A similar procedure was followed in experiment 2, with the added precaution that the experimental and control solutions were tested as "unknowns."

EXPERIMENTS AND RESULTS. In experiment 1 the following substances were tested: adrenal cortical extract, corticosterone, 17-hydroxy-11-dehydrocorticosterone, 11-desoxycorticosterone acetate and a control solution of sesame oil. In table 1 the number of rats surviving out of each group of 20 is shown in relationship to the test substance, the size and number of doses. The difference in the incidence of survival of the experimental and control groups was small and, in the case of the crystalline compounds, it is concluded that no beneficial effect was demonstrated.

In the case of adrenal cortical extract the results were suggestive of an increased resistance to shock. However, since this extract contained traces of

TABLE 1

Relationship of treatment to the numbers of rats surviving out of each group of 20 animals

DOSE			TREATMENT				
Extract (gland equivalent)	Crystals	Number doses	Adrenal extract	E	Cortico	Doc-Ac	Sesame oil control
grams	*mgm.*						
20	0.25	1	7	4	5	7	5
40	0.50	1	9	8	10	10	7
40	0.50	2	12	9	8	9	6
80	1.00	2	14	11	10	9	10
160	2.00	2	11	7	11	10	9

E, 17-hydroxy-11-dehydrocorticosterone; cortico, corticosterone; Doc-Ac, 11-desoxycorticosterone acetate. The term gland equivalent refers to the weight of the whole beef adrenal glands from which that dose of extract was derived.

epinephrine and was made up in physiologic saline, the favorable results may have been an artifact.

Experiment 2 was a study of an adrenal cortical extract from which all traces of epinephrine had been removed and which was given as a sesame oil solution. In table 2 the number of rats surviving in each group is shown in relationship to treatment. The results of this experiment were negative.

DISCUSSION. When the results of experiments 1 and 2 are considered together, it must be concluded that under the conditions of these experiments, the administration of extracts and pure steroids of the adrenal cortex failed to increase significantly the incidence of survival of rats in shock. It should be emphasized that there are sufficient data from studies in this laboratory to show that these extracts and steroids rapidly exert their biologic effects when administered in sesame oil and that these biologic effects last for longer periods of time than were studied here. Although in these experiments the test animals represented a homogeneous group, the experimental conditions were carefully standardized

and the comparison of matched treated and control animals was made simultaneously, the variability in incidence of survival among different groups was large. It is apparent that studies limited to the comparison of 15 to 20 pairs of test animals may give misleading results.

It has been reported by Selye *et al.* (1) and by Noble and Collip (2) that the resistance of non-adrenalectomized rats to shock caused by trauma was raised above normal by the administration of adrenal cortical hormones. There are a number of other reports on clinical and experimental observations in the literature which are relative to this problem but they will not be reviewed here. I am not aware of any data which prove conclusively that the resistance of the non-adrenalectomized animal or of the patient lacking damage to the adrenal glands can be increased by the administration of adrenal cortical hormones. A consideration of the favorable reports of non-crucial experiments is of little value since there have been a number of experiments showing negative results which have not been published. At the present time the question must be left open as to whether the adrenal cortical hormones are capable of raising the resistance to stress of animals and patients having undamaged adrenal glands.

TABLE 2

The relationship of the amount of adrenal cortex extract per dose to the number of rats surviving out of each group

SOLUTION	GLAND EQUIVALENT PER TWO DOSES	NUMBER TESTED	NUMBER SURVIVED
297	Control	20	8
298	80 gms.	20	6
297	Control	20	10
298	80 gms.	20	6
297	Control	15	0
298	80 gms.	15	9
288	80 gms.	20	7
289	Control	20	9
290	160 gms.	20	8
290A	Control	20	13
292	400 gms.	20	7
293	Control	20	7

The term gland equivalent refers to the weight of whole beef adrenal glands from which the combined (two doses) dosage per rat was derived.

SUMMARY

Normal male rats of 190 to 200 grams weight were caused to develop shock by the ligation of the two hind limbs for a period of two hours and thirty minutes.

A comparison was made of the incidence of survival for 24 hours of rats which were treated with adrenal cortical extracts and with certain steroids and rats which were given control solutions. The results were negative.

Acknowledgment. The author wishes to express his appreciation to Dr. E. Schwenk, The Schering Corporation, Bloomfield, New Jersey, for supplying the 11-desoxycorticosterone acetate, and to Dr. M. H. Kuizenga of this laboratory for supplying the corticosterone, 17-hydroxy-11-dehydrocorticosterone and the adrenal cortical extracts.

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THE ELECTROGRAM OF THE VENTRICLE OF THE TURTLE'S HEART

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Although the literature on the electric responses of ventricular muscle is extensive (see for references Katz, 1928, and Schütz, 1936) the pattern of the so-called "monophasic" records is still open to question. Indeed, in recent years the source of those records has been attributed to the damaged, rather than to the normal tissue (Wilson, Macleod, Johnston and Hill, 1933; Eyster, Meek, Goldberg and Gilson, 1938). The present study attempts an analysis of the pattern and site of origin of the ventricular electrogram of the turtle.

METHOD. After pithing, the heart of the turtle was excised. A quiescent ventricle was obtained by removal of the atrium. Three different ventricular preparations were employed. Two cuts were sometimes made along the two lateral ventricular edges, from the base to about 0.5 cm. from the apex. The muscle was then unfolded to yield a flat mass with a ridge in the center, corresponding to the apical region. It was laid with the inside surface downwards on filter paper moist with Ringer solution and attached to a board by pins. One corner was left free from fixation and was connected to a torsion-spring myograph. This preparation will be referred to as the "unfolded ventricle."

In other cases ventricular strips, about 5 mm. wide, were cut perpendicularly to the base. One end was fixed inside a moist chamber and the other was attached to the myograph. This preparation will be designated "ventricular strip."

In the majority of the experiments only electrical recording was desired. The intact ventricle was then placed on moist filter paper and fixed to a board by pins, as in the first preparation. After placement of stimulating and recording electrodes, the muscle was covered by a large glass funnel, which ensured a relatively closed moist chamber.

The electrodes were either chlorided silver needles, which rested lightly on the external surface of the ventricles, or else large chlorided silver plates connected to the tissue by an Agar-Ringer bridge followed by a moist wick.

Single or multiple electrograms were recorded by cathode-ray oscillographs. Direct-coupled amplifiers were used, so that the responses were recorded without distortion. The input impedance of the amplifiers was high (1 or 2 megohms). The records correspond therefore mainly to differences of potential. The procedure followed when recording with one lead corresponding to the stimulating cathode has been described elsewhere (Rosenblueth, Daughaday and Bond, 1942).

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When both mechanical and electrical tracings were desired, the beam of light from the myograph was sent to the back of the film which photographed the single oscillograph used in those observations.

The stimuli were condenser discharges with variable voltage and with a brief time constant (about 0.8 msec.). The stimulating cathode was always on intact tissue.

RESULTS. A. *Electrical responses recorded from intact to injured ventricle.* A characteristic electrogram recorded from an intact to a crushed or burned region is illustrated in figure 1A. The pattern is complex, that is, it exhibits several crests and troughs. As indicated by the numbers which label the tracing, four excursions in which the intact region becomes increasingly electronegative (*1*, *3*, *5*, and *7*) are separated by three positive dips, two (*2* and *4*) relatively,

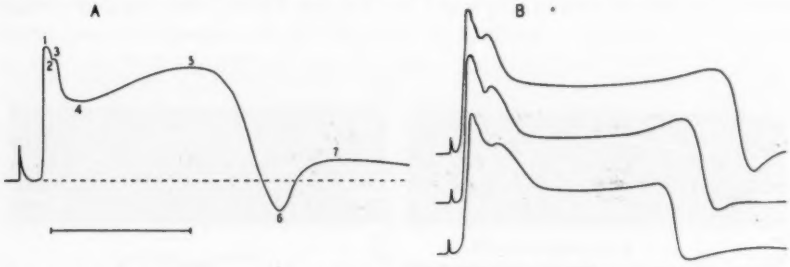

Fig. 1. Tracings of responses from leads on crushed and intact regions of a ventricle. In this and other figures with leads from live and injured tissue, respectively, upward excursions denote negativity of the lead on live tissue. The horizontal line corresponds to a time interval of 1 sec.

A. Characteristic electrogram. The numbers label the successive changes of potential differences. The record shows atypically prominent excursions *4* and *7*.

B. From above downwards, the 1st, 4th and 7th responses to a series of stimuli at the rate of 1 per 3 sec.

and the other (*6*) absolutely positive with respect to the preexisting demarcation potential.

All the waves which appear in figure 1 were not invariably present in the records. Excursion *7* was often unidentifiable. Less frequently the troughs *2* and *4* were absent. Even more rarely the positive dip *6* was absent. The records then approached the theoretical monophasic single negative wave which has often been assumed to represent the pure ventricular electrogram. Since all the excursions in figure 1 were usually recognizable, however, even when the records were taken immediately after crushing or burning the tissue, they should be included in the detailed description of a typical electrogram.

A complete electrogram, similar to that illustrated in figure 1, was found in the cat's ventricle, as follows. In three cats under dial anesthesia artificial respiration was administered. The thorax was opened by resection of the sternum. The apex of the heart was tied tightly and the ligature was fixed to a clamp, to reduce movement. The records from a lead on the tied (injured)

region to one at the base of either ventricle showed all the excursions in figure 1 as soon as tested—i.e., within 2 minutes after the injury. An additional brief diphasic excursion preceded the component *1*. This excursion is attributed to the propagation of the impulse in the conducting tissue, for it varied when ectopic beats were obtained during vagal stimulation, while the rest of the electrogram was not significantly altered.

Additional excursions were sometimes seen in the turtles. The most common was a slight initial positivity or negativity, which preceded the negative swing *1* in records taken after conduction—i.e., at some distance from the stimulating cathode. Since this initial deflection was never seen in records from the cathode, and since it was not always present in records after conduction, it is inferred that it is not an essential constituent of the electrogram.

Repetitive stimulation at a rate of 1 per 2 or per 3 secs. led to striking changes of the electric responses. As illustrated in figure 1B the responses became briefer

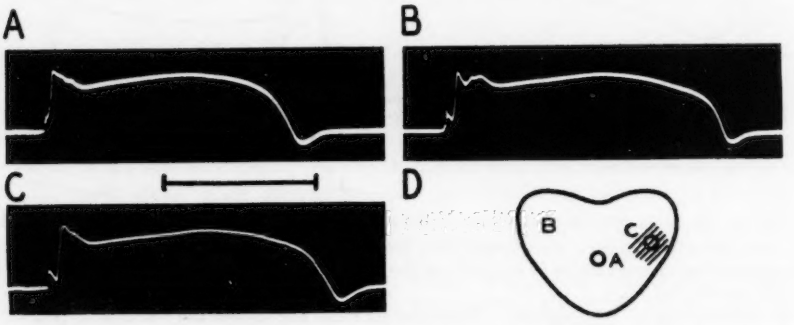

Fig. 2. Invariance of the responses obtained from an intact to a crushed ventricular region.

A, B and C, responses to stimuli with the cathode placed at the positions indicated by the corresponding letters in the diagram D. The hatched part of the diagram indicates the crushed region and the circles the position of the leads.

with successive shocks. This shortening was mainly at the expense of the late components of the electrogram, especially excursion *5*. Indeed, the early components, particularly *2* and *3*, occurred later than in the first response, and were prolonged. An early appearance of the positive wave *6* is the most striking feature of the brief responses.

The pattern of the electrograms was not significantly affected by the path of conduction followed by the propagated response in its travel towards the recording electrodes. Thus, the records in figure 2 were obtained from leads in a fixed position, but with the stimulating cathode at the points illustrated in the diagram. The responses are quite similar regardless of the region stimulated.

The latency of the responses was found to increase with the distance between the stimulating cathode and the lead on intact tissue. Since the stimulus-response delay is variable and may be quite long in the turtle's ventricle (Rosenblueth, Daughaday and Bond, 1942), the following procedure was adopted for

these observations. Simultaneous and independent records were taken from a common lead on injured tissue to each of three other leads on intact ventricle. For any given response the latency was shorter in the record from the lead nearer the stimulus. Figure 3 illustrates a typical experiment.

The latency of the responses was not influenced by the distance between the stimulated and the injured regions. Thus, in figure 4 the latency of the responses recorded from three crushed regions, respectively, to a common lead on intact tissue is the same for the three records, although the stimulus was applied close to one of the crushes and relatively far from the others.

The duration and pattern of a given response varied for several positions of the leads on live tissue (figs. 3, 7 and 10). Small, brief and atypical responses were usually recorded from leads placed at a short distance (1 to 4 mm.) from the injured region (see figs. 4B, 7B, and 10). On the other hand the amplitude

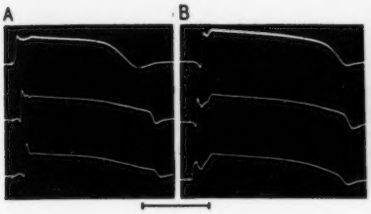

Fig. 3

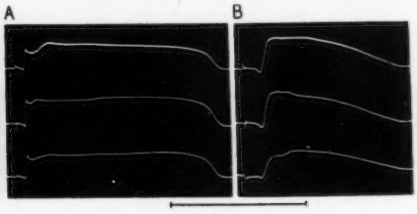

Fig. 4

Fig. 3. Independence of the records taken from a common lead on burned tissue to three different leads on intact ventricle. The live leads corresponding to the records from above downwards were 3, 6 and 9 mm. away from the burn, respectively.

In A the stimulating cathode was 1 mm. away from the lead for the top record, in B it was near the lead of the lowest record.

Fig. 4. Similarity of responses recorded from one common lead on live tissue to different leads on three separate burned regions. The stimulating cathode was near one of the burns. In A the live lead was relatively far from all the injured points, in B it was 2 mm. away from one of the burns.

duration and pattern of a given response were practically constant when a common lead on intact ventricle was tested against several crushed regions (fig. 4).

B. *Electrical responses recorded from two intact regions of the ventricle.* As opposed to the records from intact to crushed tissue, which had a similar pattern both for a given ventricle and also from one ventricle to another, the records from intact to intact tissue were quite variable. Several factors could influence their pattern. The typical record, with the two leads about 1 cm. apart and aligned with respect to the stimulating cathode, consisted mainly of two waves, one initial, the other terminal (see fig. 5). In rare simple cases each of these waves was monophasic, the first showing electronegativity and the second electropositivity of the lead proximal to the stimulus with respect to the distal lead. More commonly, however, both waves were complex, di- or even triphasic, and their polarity, particularly that of the terminal one, was variable.

The two main factors which modified the pattern of the records were the orien-

tation of the leads with respect to the wave-front of the propagated impulse and the recording interelectrodal distance. The more equidistant the two leads from the stimulating cathode, and the shorter the distance between them, the briefer the first wave of the record.

The second wave was often modified in the course of a series of responses elicited with relatively fast rate of stimulation and recorded with fixed leads. This wave then usually became briefer and its polarity could sometimes be reversed during the series. The phenomenon is illustrated in figure 6.

The polarity of the second wave was usually unchanged when stimuli were delivered first near one then near the other recording lead. In other words with fixed leads *a* and *b*, *a* could be negative with respect to *b* during the second wave whether the stimulating cathode was proximal to *a* or to *b* (fig. 5). The first wave, on the contrary, always reversed in these circumstances—i.e., the lead proximal to the stimulus was mainly negative with respect to the distal (fig. 5).

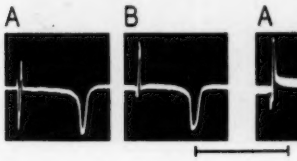

Fig. 5

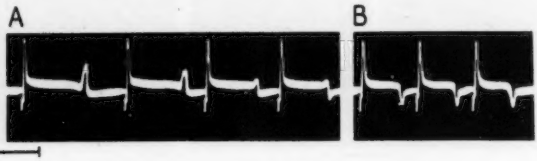

Fig. 6

Fig. 5. Changes of the records from two leads on intact tissue produced by stimulating different regions of the ventricle. One lead near the base, the other near the apex. Upward excursions denote negativity of the apical, with respect to the basal lead. At A the stimulating cathode was near the base, and at B near the apex. The stimulus artifact was too small and does not appear in the records.

Fig. 6. Changes of the records from two leads on intact tissue in the course of a series of responses. Both stimulating and recording electrodes were in fixed positions. A, beginning, and B, 8 sec. later, end of series.

Records from intact to intact tissue have commonly been attributed to the algebraic summation of the electrical events taking place under each of the leads. Figure 7 shows responses recorded between two leads on intact tissue and from these to another at a crushed region. It is quite clear that the record from live to live tissue is the algebraic sum of the other two. Indeed, when the records were projected through a photographic enlarger and corrected for the slight differences in the gain of the respective amplifiers an accurate summation was obtained.

This method afforded a means to test how strictly similar were the responses from a lead on live tissue to two different crushed regions. If the two records were quite similar no deflections should be recorded between the two leads on crushed tissue. Although as a rule there were slight differences, occasionally, as shown in figure 8, the two electrodes on dead tissue remained practically isopotential throughout a response, even when the stimulus was much closer to one of the crushed regions than to the other.

C. *So-called unipolar leads.* The method of recording from one localized lead on the surface of the ventricle to another electrode in the body of the animal or in the Ringer solution partially bathing an excised heart was used by Wilson *et al.* (1933) and by Eyster *et al.* (1938). It was considered interesting, therefore, to compare the results from this mode of recording with those reported above.

All our observations agree with the inference (see discussion below) that the external electrode is equivalent to a diffuse lead applied to a large fraction of the ventricular surface. The influence of the position of the leads on live tissue with respect to the stimulated region was less striking with the external leads (figs. 9 and 10) than with those applied to a localized region (fig. 3), as would be expected from a diffuse lead, but it was clearly recognizable.

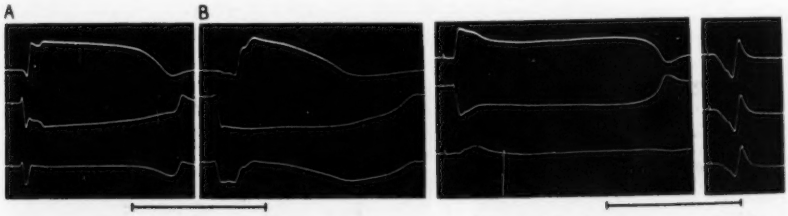

Fig. 7 Fig. 8 Fig. 9

Fig. 7. Algebraic summation of two records from one injured to two intact regions yields the record from intact to intact regions. The two upper records correspond to a lead at a burned region and each of two separate leads on live tissue. The lower record is from the two leads on intact tissue. In A both live leads were well away from the burn. In B the lead corresponding to the upper record was near the burn.

Fig. 8. Negligible differences of potential recorded from two leads on burned ventricle although the stimulus was applied close to one and far from the other burn. The two upper records are from each of the burns to a common lead on live tissue. The lower record is from the two burns.

Fig. 9. Differences between records obtained from a common localized lead on live ventricle to 3 different electrodes placed in contact with Ringer solution bathing the heart partially. For explanation see text.

In figure 9, three external leads are tested against a single localized lead on live tissue. Only the beginning of the records is shown. The upward excursion, simultaneous in the three records, signals the arrival of the impulse at the localized lead. The external lead corresponding to the top record was on the side of the heart proximal to the stimulated region. The initial downward excursion is more prominent in this record. The lowest record is from the external lead on the opposite side of the ventricle. The initial downward excursion is slow and small. The middle record, from an external lead in an intermediate position, shows likewise intermediate features of the first excursion.

In figure 10 a lead on burned tissue is referred to an external lead (top record) and to two localized leads on intact tissue, one (middle record) 3 mm. distant from the burn, and the other (lowest record) 6 mm. away. The stimulus was applied 15 mm. away from the burn. The three records differ in latency, in pattern and in duration.

D. *The influence of veratrine on the electrogram.* The negative after-potentials of nerve (Graham, 1930), of the superior cervical ganglion (Rosenblueth and del Pozo, 1942a) and of striated muscle (Rosenblueth, Wills and Hoagland, 1941), are all markedly increased by veratrine. Observations were made, therefore, to see whether any of the components of the ventricular electrogram would be similarly increased by this drug, thus indicating an analogy with the negative afterpotential of the structures mentioned.

Veratrine (0.5 to 1 mgm.) was injected into the atrium in some animals, before excision of the heart. Although the doses of the drug used were sufficient to cause the appearance of rhythmic activity in some of the ventricles tested, in no case was there a significant increase of any of the components of the electrogram. The records from the veratrinized ventricles were quite similar to those obtained from the normal muscles.

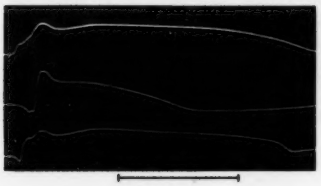

Fig. 10

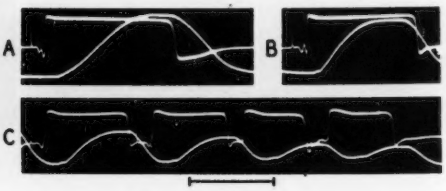

Fig. 11

Fig. 10. Differences in the records from a lead on burned tissue referred to an external lead on the Ringer solution partially bathing the preparation (top record) and to two localized leads on intact tissue, respectively. One of the latter electrodes (middle record) was 3 mm. away from the burn, the other (lowest record) 6 mm. away.

Fig. 11. Changes in the electrical and mechanical responses in the course of a series of stimuli. Unfolded ventricle (see Method). Upper record: electrogram; lower record: isometric mechanogram. One of the recording leads was on the part of the ventricle which registered mechanically, the other lead was on a crushed part, 5 mm. away. A, 1st response; b, 4th response; and C, the last 4 responses in a series of 10.

Similarly negative results were found in the three cats studied (p. 465). Injections of veratrine (1 to 2 mgm. per kgm.) did not modify the electrograms significantly.

E. *The relations between the electrical and mechanical responses.* Since this is a point which has been abundantly studied (see Schütz, 1936), only incidental observations were made, with the limited purpose of seeing whether any of the components of the electrogram would show variations parallel with those in the mechanical events.

Efforts were made to obtain the electrical records from the region of the ventricle which was registering mechanically. This was not feasible in the strips of muscle, since the electrical record was relatively localized, whereas the whole strip contributed mechanically. In the unfolded ventricles (see Method), on the other hand, the contractions recorded were mainly those from a small part of the ventricle and the electrical responses were led from one electrode on that part and the other about 0.5 cm. away on a crushed region.

The beginning of contraction coincided usually with the excursion *3* (fig. 1) of the electrogram. There was no correlation, however, between the beginning of relaxation and any of the electrical events. Indeed, when a series of beats was elicited at rates of 1 per 2 to 1 per 5 secs. the electrical and mechanical responses varied independently. As shown in figure 11, both electrical and mechanical events became briefer, but the contractions shortened more at first whereas the electrograms shortened more than the mechanograms later in the series.

The only electrical phenomenon that showed any correlation with the amplitude of contraction was the positive swing *6* (fig. 1). This correlation was very striking in the only two ventricles studied which exhibited alternation of contractions when stimulated at a rate of about 0.7 per sec. The large mechanical responses coincided with a large *6* in the electrogram, whereas the small contractions were attended by only a small *6* (fig. 12).

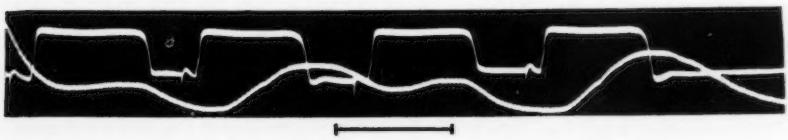

Fig. 12. Alternation of the mechanical responses after veratrine (0.5 mgm. into the auricle, before removal of the heart). Lower tracing: mechanogram. Upper tracing: monotopic electrogram. Note the alternation of component *6* (see fig. 1). The record begins with the second stimulus in a series.

Discussion. I. *Some problems of electrical recording.* The terms monophasic and diphasic are often employed to distinguish records obtained from intact to damaged tissue and those led from two intact regions. As has often been pointed out, these terms are ambiguous. Strictly speaking a monophasic record is one exhibiting a single unidirectional wave. No tissue has so far yielded such a record. The so-called monophasic record of nerve exhibits several components, some negative, some positive (see Gasser, 1937); it is therefore not a monophasic, but a polyphasic record.

A fundamental question in the study of the electrical behavior of any cell or tissue is the determination of the potential changes during activity at an ideally isolated small region. These changes can be referred to a local system of coordinates, e.g., from inside to outside the cell, and also to an ideal indifferent lead on inactive tissue. The term monopolar, often used to characterize a mode of stimulation, might be used to describe the records which would answer that question. But the term monopolar is a misnomer, for records, like stimuli, require two poles—two electrodes. We propose, therefore, to use the term "monotopic" to designate a record which is representative of electrical events at a small region of a tissue.

II. *The site of origin of the electrical changes recorded from an intact to an injured region.* The classical view is that those records are due to changes in the intact region of the ventricle to which the lead is applied. As already mentioned,

however, Wilson *et al.* (1933) and Eyster *et al.* (1938) have suggested that the source of the potential changes is the damaged region.

This suggestion is based mainly on observations made with so-called unipolar records. As pointed out (see figs. 9 and 10), however, this procedure is equivalent to leading directly from one localized to another diffuse lead on the ventricle (p. 469). The external lead is not an indifferent lead. At best it indicates the average potential, but this average can fluctuate widely depending on the electrical events in the tissue.

The following arguments are against the view that the injured tissue, or the immediately neighboring regions, are the source of the responses recorded. The same arguments support the opposite view that the source is the intact muscle. It is highly improbable that dead tissue should respond, even when attached to live tissue. In analogy with what occurs in nerve, one would expect not only that the dead tissue should be inactive, but also that the muscle in the neighborhood of the injured region should be depressed. In accord with these expectations, when records are taken with two localized leads, the closer the electrode on live tissue is to the injury, the smaller and briefer the response (see figs. 4B, 7B and 10).

If the responses with localized leads from injured to intact regions were mainly due to events at or near the damaged area (Eyster *et al.*, 1938), then the records from one injury to different intact points should have a constant latency and duration, regardless of the point stimulated, but this is not the case (figs. 3, 7 and 10). On the other hand, significant differences of latency and duration would be expected when leading from several injured regions to one intact portion, but this is also not a fact (figs. 4 and 8). When the stimulus is applied near one of several injured portions and leads are taken from two of them, asynchronism would cause marked deflections in the record if the source of the responses were at the injury, but the records exhibit only minor differences of potential (fig. 8). Finally may be mentioned the report of Schütz (1936, p. 515) that local warming of the injured region has no influence on the recorded responses, while local warming of the intact region to which a lead is applied causes the responses to become markedly briefer.

It is inferred, therefore, that in the heart, as in nerve and skeletal muscle, the electric responses originate from intact, not from damaged parts.

III. *The monotopic character of the records from intact to dead tissue.* Although, as shown above, the electric responses originate from intact tissue, the previous argument does not answer the question of what parts of the tissue contribute to the records—i.e., whether it is only the intact region in contact with the lead that records, or whether other uninjured portions of the ventricle are also contributory.

That the records are mainly monotopic, from the intact portion tapped, is shown by the following data. The independence of latency, amplitude and duration of records from different live spots (fig. 3) is in favor of that view. The invariance, except for latency, of responses recorded from fixed leads and variable stimulated regions supports also the view (fig. 2). Finally, Schütz's (1936)

observations on the action of local heating, cited above, are favorable to the inference.

IV. *The components of the monotopic response.* It has often been assumed that this response should be strictly monophasic—i.e., should consist of a single, simple, unidirectional excursion—and that any deviations from that simple tracing are due to artifacts. The actual records seldom, if ever, conform with this ideal pattern. Their complexity has been recognized by the distinction of two components (see for references Katz, 1928, and Schütz, 1936). To account for the 7 excursions illustrated in figure 1, at least 4 components must be assumed, if those excursions are not artifacts.

Spurious waves could be due to two factors: movement artifacts and an asynchronous contribution of similar, relatively simple events at different regions of the muscle, specifically the regions in the neighborhood of the lead on crushed tissue (so-called diphasic artifacts).

The early part of the electrogram (excursions *1* and *2*) preceded the mechanical response if the lead on intact tissue was sufficiently close to the stimulating cathode. This part of the electrogram took place before the propagated impulse reached the neighborhood of the lead on crushed tissue if the recording interelectrodal distance was large and the stimulus was applied far from the crush. Since records similar to that in figure 1 were obtained with relatively large interelectrodal distances (about 2 cm.) and with stimulation at or near the lead on intact tissue it may be inferred that the early part of the electrogram is free of artifacts from the two sources mentioned.

Both movement and asynchronism should be modified if, with a fixed position of the recording leads, the cardiac responses were started at different points by moving the stimulating electrodes. Hence, if the two sources of artifact mentioned were significant, shifts of the stimulating electrodes should significantly modify the recorded electrogram. It was found, however, that the electrical responses did not as a rule vary when the stimulated region was varied (fig. 2).

Movement artifacts may occur. Thus, it is likely that the initial deflection seen sometimes in responses recorded after conduction (p. 466) was due to movement. The responses from a given pair of leads usually changed slowly in the course of time, the main feature being the accentuation of the positive component *4*. Further crushing or burning in the injured region then restored the original pattern. This change with time is therefore probably due to progressive healing of the injury—i.e., it constitutes a "diphasic" artifact.

Since all the components in figure 1 were usually present within 1 minute after injury and in approximately isometric conditions, it is concluded that these components are genuine constituents of the monotopic electrogram. It would be surprising if the ventricular response were simple, for in all the tissues studied so far—nerve (see Gasser, 1937), striated muscle (Rosenblueth, Wills and Hoagland, 1941) and smooth muscle (see Rosenblueth and del Pozo, 1942b)—the electrogram is complex.

The conducting mechanism in the turtle's ventricle is similar in its properties to that in nerve and in striated muscle. The first component (*1*) in the ventric-

ular electrogram may therefore be analogized with the spike potential of the other tissues. Since the positive component *6* of the ventricle was found to vary with the degree of contraction (fig. 12), this component may be analogous to the positive component which varies in striated muscle with contraction (Rosenblueth, Wills and Hoagland, 1941). No further analogies may be drawn, however, with the data available. A puzzling item of negative information is the relative indifference of the ventricular electrogram to veratrine. Since in other tissues (see p. 470), a spike potential is followed by a negative afterpotential which increases dramatically with veratrine, the data on the heart may imply that the ventricle lacks this type of negative afterpotential. It is possible, however, that this potential may exist, but that even when increased by veratrine it is masked by other, more prominent, negative components (*3* and *5*).

V. *The records from intact to intact regions.* Since the records from live to injured regions are monotopic and correspond to the live point, it follows that the records from live to live leads are ditopic and correspond to the algebraic summation of the events at each of the two leads. This corollary is illustrated in figure 7. It should be pointed out, however, that an accurate algebraic summation of the potential changes at several electrodes forming a closed loop (fig. 7; see Eyster *et al.*, 1938) does not prove anything with regard to the source of the potentials recorded. What it proves is merely that Kirchhoff's e.m.f. law applies to the cardiac circuit, much as it applies to any other circuit which has been tested so far. In other words, the potential changes between any two electrodes on the heart will always be the algebraic sum of the changes between these two leads and any number of other electrodes, so arranged that they form a closed loop. The applicability of Kirchhoff's law does not give any information about the location of sources of differences of e.m.f.

The analysis of the factors which determine the terminal wave of the ditopic elelectrogram is of significance for the interpretation of the wave T of the electrocardiogram. The data indicate that the most important factor is the asynchronous termination of the electric responses at the two recording regions. This asynchronism might be due to the discrepancy of the beginning of the response at the two points, because of the time lost in the conduction of an impulse from one to the other. The results suggest, however, that conduction time is a relatively unimportant factor for the determination of the pattern and polarity of the terminal wave (figs. 5 and 6). The most important condition, apparently, is the difference in the duration of the action potentials at different regions of the ventricle. Not only is this duration variable for different regions, but it may vary independently at each of them in a series of responses (fig. 6).

The complexity and variability of pattern of the terminal wave of ditopic records is readily understood since the terminal part of the monotopic records is itself complex (components *5*, *6* and *7*). The algebraic summation of two asynchronous simple waves would lead to a relatively simple pattern, but the summation of complex events can lead to an intricate sequence of potential changes.

VI. *The relations between the electrical and mechanical events.* Although the

observations on the mechanical responses were only incidental, some comments are justified by the data obtained.

The correlation between the time course of mechanical and electrical events in figure 11 can only be approximate, because the electrical record is monotopic whereas the mechanical record corresponds to a relatively large portion of muscle whose elements respond asynchronously. Notwithstanding this lack of accuracy, the changes during a series of responses support the conclusion that the electrical and the mechanical phenomena are largely unrelated. Many other lines of evidence lead to the same conclusion (see Schütz, 1936).

Blair, Wedd and Young (1941) have proposed the hypothesis that the contractile process is released by the depolarization wave consequent to excitation and proceeds as long as the membrane remains depolarized. The contractile process would be retarded or arrested when the membrane begans to repolarize. The arrest would be complete by the time repolarization is complete. Thus polarization would govern contraction, not contraction polarization. The present observations do not support this hypothesis. If polarization (i.e., negativity of the active region) controlled contraction, then there should be a strict temporal correlation between the end of that negativity (component *6*) and the beginning of relaxation. But figures 9 and 10 show that this correlation does not exist.

It is concluded that the potential changes and contraction are largely independent. The spike potential (component *1*) may not only be the agent for conduction but may also trip the contractile process. The time course of contraction is probably governed by other factors than the potential changes. It is likely that one (component *6*) or more of the excursions in the electrogram (fig. 1) are the electric manifestation of the physicochemical events associated with contraction; in that sense contraction may control polarization. It is also likely, however, that several of the electrical changes denote processes irrelevant to contraction—i.e., they may be associated with conduction and its recovery cycle.

SUMMARY

Evidence is presented (figs. 2, 3, 4, 8, 9 and 10) which indicates that the electric phenomena recorded from leads on an intact and an injured region of the turtle's ventricle are due to changes which occur in the intact, not the injured part of the muscle (p. 472). This record may be called monotopic, to avoid the use of other ambiguous terms (p. 471). This electrogram exhibits several components (fig. 1A), which may vary independently (figs. 1B and 12). The significance of some of these components is discussed (p. 473).

The records with leads from intact to intact tissue are influenced by the position of the leads with respect to the stimulated region (fig. 5). They may also be modified in the course of a series of responses (fig. 6). These records may be called ditopic, since they represent the algebraic summation of two monotopic records (fig. 7, p. 474).

The electric and the mechanical phenomena of the ventricle are largely independent (fig. 11, p. 475).

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TONUS CHANGES IN CARDIAC MUSCLE AND THEIR SIGNIFICANCE FOR THE INITIATION OF IMPULSES

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In a previous paper non-conducted potential changes were described which can initiate impulses and are responsible for the automaticity of cardiac muscle (2). Here it will be shown that these electric changes are accompanied by active changes in tension which, like the former, are non-conducted and graded. Because they are quite distinct from the all-or-none responses they will be called tonus changes, but they have nothing in common with the well-known tonus waves of the turtle's auricle.

METHODS. Most experiments were carried out with the ventricles of turtles. Responses were elicited by induction shocks. The contractions of muscle strips (3 to 4 mm. wide, 1 cm. long) were recorded by a very sensitive isometric lever on a smoked drum or photographically. The preparations were immersed in Ringer's solution except for the upper tip to which one of the stimulating electrodes was attached. The responses of the whole ventricle were recorded under isometric conditions by a membrane manometer on a smoked drum. The manometer was connected to the ventricle by a glass tube inserted through the auricle. The A-V valves were removed and the large arteries were tied off at their origin. The whole system was filled with fluid and a side tube was provided for regulating the initial pressure. The natural period of the whole system was 12 vibrations per second. The sensitivity of the manometer was high at low pressure and decreased at higher pressures which made the instrument particularly suitable for recording weak tonus changes.

For simultaneous recording of tension and potential the muscles were suspended in a shielded chamber above the muscle lever. The upper end of the muscle was inside a glass tube so that monophasic potentials could be recorded (1, 2).

RESULTS. The tonus changes following a conducted response were studied in two series of experiments. In the first series, the contractions of strips from the ventricle were recorded by an isometric lever. In the second series the contractions of the whole ventricle were recorded by a manometer. The results of both series were essentially the same, showing that mechanical injury like that involved in the preparation of the strips is not a necessary condition for the tonus changes. It was found that the tonus changes are very similar to the after-potentials previously described, a rise in negativity corresponding to a rise in tonus and vice versa.

In a fresh preparation the tension of the muscle drops below the previous resting level after each contraction (fig. 1A, 2A). This phenomenon, which corresponds to the positive after-potential present under the same conditions

(2), is significant because it shows that a weak tension is actively maintained even in resting muscle. The transitory drop in tonus was found in all experiments with muscle strips although its magnitude was very variable. It was absent in two of the twelve whole ventricles used and in two others it was found only when the initial pressure was higher than 6 mm. Hg. The changes in ten-

Fig. 1. Tonus changes following responses of a strip from the turtle ventricle recorded by an isometric lever. A. Muscle in Ringer's showing decrease of tonus below previous resting level and slow return after response. B. Same muscle in mixture of equal parts of 1 per cent $CaCl_2$ and Ringer's. Temp. 23°. Calibration 2 grams. Time seconds.

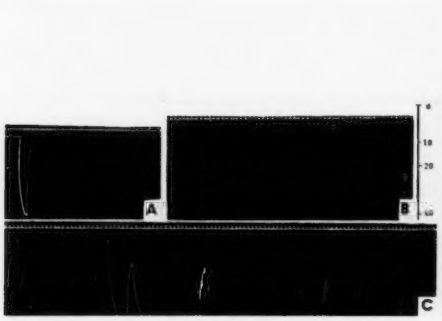

Fig. 2

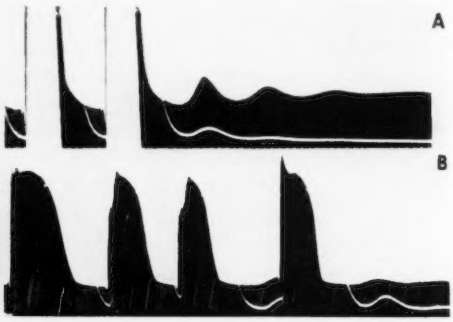

Fig. 3

Fig. 2. Tonus changes of a whole ventricle recorded by a manometer. A. Ventricle filled with Ringer's, showing decrease of tonus after response. B. Ventricle filled with equal parts of 1 per cent $CaCl_2$ and Ringer's showing oscillatory after-effect. C. Same as B but with higher initial pressure giving a slower relaxation. Calibration: mm. Hg pressure. Note decrease in sensitivity of manometer at higher pressures. Time seconds.

Fig. 3. Oscillatory after-potentials and tonus changes recorded simultaneously from a strip of the turtle's ventricle. Upper curve oscillograph record, lower curve isometric lever. All-or-none responses were elicited by induction shocks except the last response in B, indicated by +, which was initiated by a local potential and was preceded by a tonus change. Time $\frac{1}{5}$ second.

sion or pressure following the conducted responses were always very small, but no mechanical defect of the recording system was found which could account for the observed phenomena. Slipping or leakage would have produced a drop in tension or pressure, but could not explain the subsequent rise. The lever used had a small elastic after-effect which tended to diminish the effect recorded.

After treatment with a large excess of calcium ions the tonus becomes elevated

and relaxation is slower than normally, particularly at high initial tensions. In some cases, the tension drops markedly below the previous level after a response. The most striking effect of this treatment, however, are rhythmic variations of pressure or tension which decrease in a regular manner and disappear after 1 to 5 waves exactly like the oscillatory after-potentials described previously (fig. 1B; 2B, C; 3). The electric and mechanical changes run closely parallel but, as expected, the former always slightly precede the latter (fig. 3). Tonus oscillations have also been recorded from preparations of the sinus venosus. They are quite distinct from the tonus waves caused by smooth muscle.

Because of technical difficulties it has not been tested whether the spontaneous prepotentials such as they occur in the sinus venosus are accompanied by tonus changes. However, this is probably the case because there does not seem to be any essential difference between pre- and after-potentials and because in the ureter the tonus changes accompanying spontaneous prepotentials have been demonstrated.

Discussion. It is not intended to give a full discussion of the question of tonus in cardiac muscle. The term is generally used to describe changes in "resting length" or, in other words, slow contractions which are sharply distinguished from all-or-none responses. Several investigators have claimed to have demonstrated such a phenomenon in cardiac muscle, but it appears that the underlying observations can be explained as the result of variations in the completeness of relaxation between beats caused by changes in heart rate or in the strength and duration of each beat (cf. 4). Such an interpretation cannot be applied to the tonus changes described above because single responses of quiescent muscles were studied.

It has been shown here that a small tension is actively produced and maintained even in the "resting" muscle. Obviously, this tension could not have been distinguished from passive elastic tension as long as it remained constant. The presence of a tonic contraction, therefore, cannot be detected by a study of the mechanical properties of the muscle alone and the question whether a similar condition exists also in other striated muscles can only be decided by further studies.

The tonus changes described may be considered as changes in the "resting" tension of the muscle. There is no reason, however, to assume a fundamental difference between this type of contraction and all-or-none responses. Both are accompanied by potential changes. The difference probably lies in the excitatory mechanism and chiefly in the presence or absence of conduction. That the tonus changes involve the production of mechanical energy is shown most strikingly in the tonus oscillations. It must be assumed, therefore, that tonus changes are caused by changes of metabolism. A simple explanation of the phenomenon would be the assumption that the resting metabolism involves the contractile mechanism and thereby causes the production of a small amount of mechanical energy. This assumption, according to which there is only a quantitative difference between activity and resting metabolism, is not in disagreement with our present knowledge of the chemistry of these processes.

The oscillations of tonus and electric potential undoubtedly express an important property of cardiac muscle, but their nature is obscure. Although they resemble oscillations of simple systems, a purely physical explanation based on mechanical and electric forces operating at the cell surface, such as the hypothesis considered by Cole (3) for potential oscillations in nerve fibers, appears untenable. The fact that in muscle mechanical changes are associated with the oscillations shows that they do not involve merely the cell surface. Their low frequency, several hundred times smaller than in nerve fibers, adds another difficulty for the application of Cole's hypothesis to muscle. The frequency in the various tissues studied so far appears to be correlated with the time relations of their responses. The phenomenon is perhaps comparable to chemical oscillations.

SUMMARY

The after-potentials of cardiac muscle previously described are accompanied by changes in "tonus". In fresh muscle the tension drops slightly below the previous level corresponding to the positive after-potential observed under the same conditions. This phenomenon is significant because it indicates the presence of a tonic contraction in "resting" muscle. After treatment with an excess of calcium ions the responses are followed by oscillatory changes in tension.

The tonus changes and the local potentials are probably manifestations of a more fundamental process, a fluctuation in resting metabolism. The mechanical changes are weak and hardly play any rôle as such. Their chief interest lies in their relation to the automaticity and rhythmicity of the muscle. It may be assumed that an increase in metabolism causes a rise in tonus and a decreased surface polarization. The decrease in polarization in turn may be considered as the last link in the chain of processes leading to the discharge of an impulse.

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